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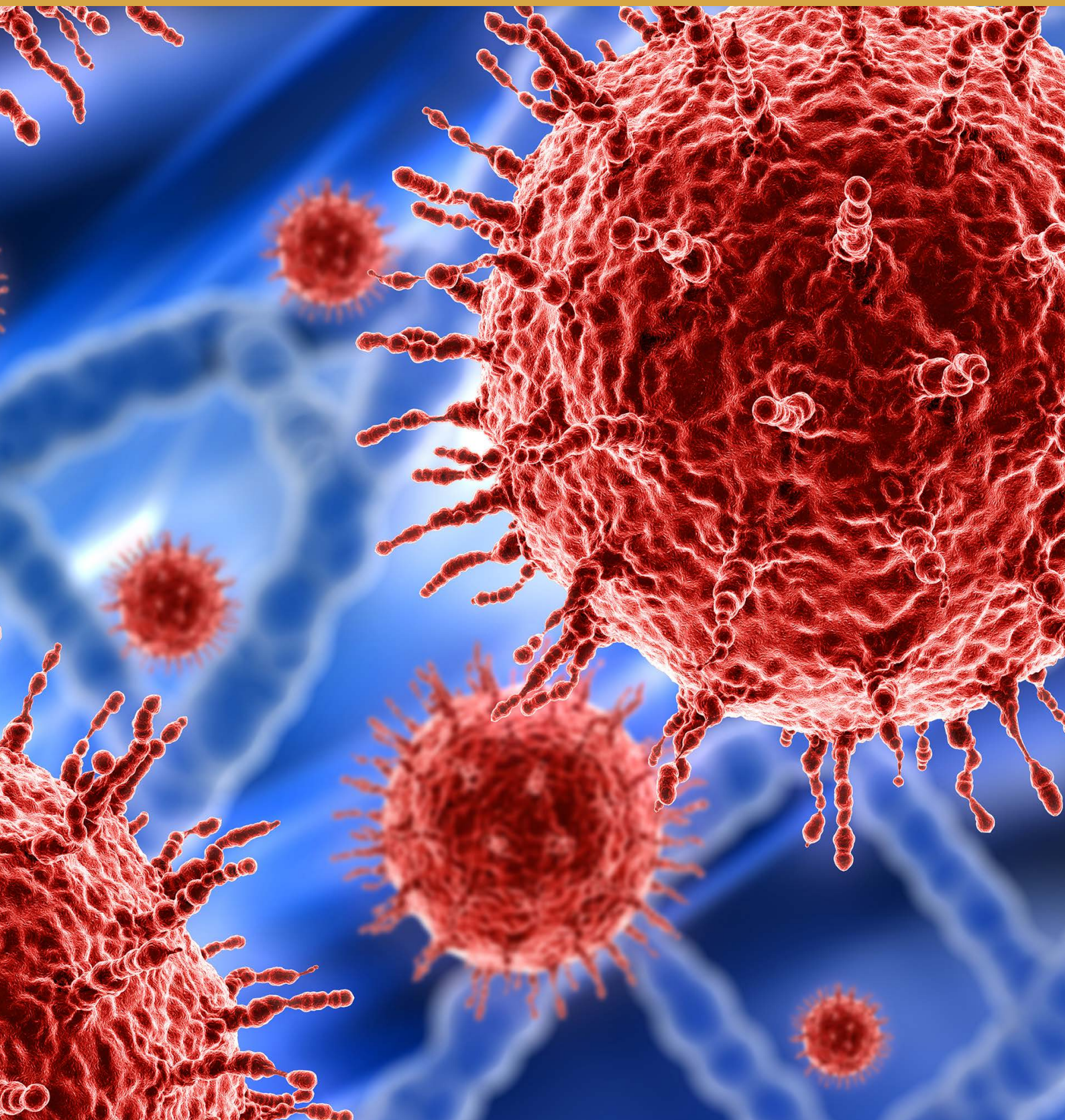

Contents

This volume includes five chapters dealing with some of the latest advances in medicine and biology. Chapter one pontificates on the impact of the sequencing of the human genome on the search for life in the universe. Chapter two summarizes current understanding of how to assess endothelial dysfunction, main risk factors, mechanisms underlying its development, prognostic implications, and biomarkers. Chapter three assesses the effects of the hormone irisin on glucose metabolism. Chapter four highlights the common disorders that need to be considered as a differential diagnosis for neuromyelitis optica spectrum disorder and features that help in their differentiation. Finally, chapter five deals with tapered modular femoral components in revision total hip arthroplasty.

Chapter 1 - This abstract describes how Sir Isaac Newton's dream was converted into reality. In 1718, he predicted that one day we will convert Bodies into Light and reconvert Light into Bodies. That day is approaching fast. When we decoded the Human Genome and learned that the language of Life of all Life forms is written in DNA which carries two binary codes of Nucleotide and they are A (Adenine), T (Thiamine) and G (Guanine) C (Cytosine). These four nucleotides form the Analog Language of Biology could easily be converted into the Digital Language of Computer. The digital language is written in a binary code, and they are Ones and Zeros. The Genomic codes converted into the Digital codes could travel with the speed of Light to every corner of the Universe and these signals could be re-converted back to the nucleotide code of Life. For example, Mars's rover Curiosity is equipped with the Genome sequencer onboard. If it succeeds in extracting DNA from Martian micro-fossil, it could sequence its DNA. Once it reads the Genetic code, it could convert to Digital Code and send its sequence data to Earth with the speed of Light. Within four and a half minutes, we could receive the Digital Code and re-convert to its Genetic Code. By using the nucleotides available in the Lab, we could recreate the structure and determine what kind of microbial Life if ever existed on planet Mars. Although we have succeeded in sending and receiving information with the speed of Light, sending matter is still a dream waiting to be accomplished. Teleportation is the hypothetical transfer of matter or energy from one point to another without traversing the physical space between them. With this knowledge, we could send future spacecrafts equipped with sequencer/converter to any one of those recently discovered 4,000 exoplanets in the Milky Way Galaxy to study the atmospheric and surface composition of an exoplanet to find a habitable planet for humanity.

Chapter 2 - Evaluation of endothelial dysfunction (ED) as a systemic disease has gained increasing attention in view of its emerging relevance for cardiovascular control. ED as an important early sign of generalized vascular disease contributes tomost forms of atherosclerotic cardiovascular disease (CVD) including hypertension and chronic heart failure. ED reflects a vascular phenotype prone to the initiation and progression of atherogenesis and its complications. Atherosclerosis begins in childhood and ED as the precursor and earliest clinically detectable form of atherosclerosis can be demonstrated already in early childhood, through a long preclinical stage. The authors discuss future considerations, such as unifying mechanisms in numerous pathophysiologic processes that participate in ED with major clinical implications. The authors herein summarize current understanding of how to assess ED, main risk factors, mechanisms underlying the development of ED, prognostic implications of ED, biomarkers that reflect ED, which have been proposed to indicate ED and/or vascular damage, respectively. The current normative data on the possibilities of early non-invasive detection of ED with a focus on the currently widely used method of plethysmography with its strengths and limitations are mentioned. A new option for a non-invasive determination of ED is a combined diagnostic approach by measuring the plethysmographic flow-induced Reactive Hyperemia Index (RHI) using EndPAT index as the ratio of endothelium-dependent postocclusive and preocclusive vascular tone changes. In this regard, circulating ED markers include asymmetric dimethylarginine (ADMA)-related biomarkers, high-sensitive CRP (hsCRP), vascular adhesion molecule-1 (VCAM-1) and E-selectin. Significantly decreased RHI with elevated plasma concentrations of biomarkers imply a possible association with premature ED in young patients and confirm the progression of atherosclerosis into adulthood. Microvascular ED has been associated in young patients not only with metabolic syndrome, obesity, hypertension, diabetic vascular complications, dyslipidemia or atherosclerosis but also with many different disease processes and risk factors including chronic inflammatory, autoimmune and widespread systemic diseases, infection, sepsis, hemolytic-uremic syndrome, rheumatic diseases, celiac disease, Henoch-Schonlein purpura, Kawasaki disease, low birth weight, sleep-disordered breathing, leukaemia, beta-thalassemia major, idiopathic pulmonary hypertension, and so on, without overt CVD. Especially in pediatric cystic fibrosis (CF) patients, the authors' group demonstrated the progressive development of microvascular ED from childhood to adulthood. Better understanding of a close relationship between the underlying diseases, ED and CVD, early identification, the potential discovery of therapeutic targets and the restoring of ED could serve as a preventive approach to protect against the occurrence of ED and the subsequent complications such as atherosclerosis in "at risk" paediatric population. The combined approach seems to improve risk prediction for the earliest assessment of ED before classically recognized markers of disease, and may be useful for the further clarification of premature atherosclerosis and future CVD.

Chapter 3 - Given its increasing prevalence, obesity is now considered a pandemic. Several bioactive molecules, such as cytokines, acute phase proteins and hormones secreted throughout the body are involved in the development of obesity. The hormones secreted by the white adipose tissue mass that increases with obesity are known to be effective also in the glucose metabolism disorder associated with obesity. In addition, irisin, a muscle-derived hormone that is also secreted by adipose tissue, is considered a significant agent in the prevention/treatment of obesity and the resulting glucose metabolism disorder, with its ability to convert white adipose tissue into brown adipose tissue. In this book chapter the authors assess the effects of the hormone irisin on the glucose metabolism.

Chapter 4 - Neuromyelitis Optica Spectrum Disorder is now considered an autoimmune astrocytopathy causing demyelinating and necrotizing disease of the CNS. With many differentials under the rubric of demyelinating disorders affecting the central nervous system, recent research has proven NMOSD to have a distinct pathological and clinical presentation. This also has treatment implications especially in other demyelinating disorders like Multiple Sclerosis wherein certain therapies for MS may prove to be detrimental for patients with NMOSD. Also, patients may present with disorders that have a similar phenotype with NMOSD. Since this includes a host of aetiologies like inflammatory, infective, vascular and malignant diseases, it is imperative for the clinician to rule them out systematically by clinical, serological or radiological features. This review will highlight the common disorders that need to be considered as a differential diagnosis for NMOSD and features that help in their differentiation.

Chapter 5 - The burden of revision total hip arthroplasty continues to increase. These cases are often associated with compromised femoral bone stock making conventional prosthesis unsuitable. While this remains a technically challenging procedure, modular tapered femoral components are valuable tools in dealing with problems unique to femoral revision cases. Distal fixation bypasses proximal bone defects with predictable biologic fixation. Modularity allows more precise restoration of femoral offset and leg length, as well as correction of preexisting rotational deformities. However, the addition of a stem-proximal body junction increases the risk of failure due to fretting corrosion and fatigue fracture. Modular femoral components have added more options for revision total hip arthroplasty to improve stability and allow for osseointegration, but there remains a need for larger studies examining longer term outcomes greater than ten years for these components.

Chapter 1

Space Medicine: The Impact of Sequencing Human Genome on The Search for Life in The Universe

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ABSTRACT

This abstract describes how Sir Isaac Newton's dream was converted into reality. In 1718, he predicted that one day we will convert Bodies into Light and reconvert Light into Bodies. That day is approaching fast. When we decoded the Human Genome and learned that the language of Life of all Life forms is written in DNA which carries two binary codes of Nucleotide and they are A (Adenine), T (Thiamine) and G (Guanine) C (Cytosine). These four nucleotides form the Analog Language of Biology could easily be converted into the Digital Language of Computer. The digital language is written in a binary code, and they are Ones and Zeros. The Genomic codes converted into the Digital codes could travel with the speed of Light to every corner of the Universe and these signals could be re-converted back to the nucleotide code of Life. For example, Mars's rover Curiosity is equipped with the Genome sequencer onboard. If it succeeds in extracting DNA from Martian micro-fossil, it could sequence its DNA. Once it reads the Genetic code, it could convert to Digital Code and send its sequence data to Earth with the speed of Light. Within four and a half minutes, we could receive the Digital Code and re-convert to its Genetic Code. By using the nucleotides available in the Lab, we could recreate the structure and determine what kind of microbial Life if ever existed on planet Mars. Although we have succeeded in sending and receiving information with the speed of Light, sending matter is still a dream waiting to be accomplished. Teleportation is the hypothetical transfer of matter or energy from one point to another without traversing the physical space between them. With this knowledge, we could send future spacecrafts equipped with sequencer/converter to any one of those recently discovered 4,000 exoplanets in the Milky Way Galaxy to study the atmospheric and surface composition of an exoplanet to find a habitable planet for humanity.

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Historical Background

Since the dawn of human civilization, we have asked ourselves some of the most amazing and mysterious questions which have faced us for centuries; Who are we? Where have we all come from? What was it that made us this way? How did the Universe begin? Why is it expanding at an accelerating speed? How is it likely to end? Are we alone in the vast Universe? Or are there other creatures who may live in the deep dark space who may or may not look like us? Who else live out there? What is Life? How did Earth form? How did life originate and populate our planet? Is there life out there? These questions have been around for thousands of years, for at least from the time of the Greek philosophers, I am here to tell you how closer we are getting to finding out the answers to some of these questions.

This article attempts to answer two important sets of questions in science (1) an ancient set of questions and (2) another the modern set. The ancient set includes: what is Life, and can it be created in the Lab? Second set includes, can Matter be converted to Light and reconvert Matter back to Light.

In 1943, Erwin Schrodinger was the first to ask the question, "What is Life?" Now we know that Life consists of DNA driven biological machine. Using the basic building blocks of DNA which are the four nucleotides and restriction enzymes and a computer programs, we synthesized Life in the Lab. Synthetic biology seeks to free Life from the shackles of evolution creating new organism with designer genes within weeks not in millions of years. The first step in understanding Life is to take it apart. Then the next step is to put it back together and finally to check if it functions properly to prove that you have not neglected anything by building Life from scratch. This landmark discovery has not only remarkable technical feat drawing upon the work of decades of discoveries in molecular biology, but also has placed humankind at the threshold of the most importance and exciting phase of biological research. With ability to write the software of Life; we now have the knowledge not only to guide our own future development but also to design new species as to help us adapt and evolve for our long-term survival. The synthetic microbial Life will carry instructions to produce new food, new fuel, and new medicine to treat every disease known to mankind.

In 1718, Sir Isaac Newton asked the second question. Can Bodies be converted into Light and reconvert Light back into Bodies? This article describes the synthesis of these two great ideas when we learn to convert analog language of Biology into digital language of computer. Once we convert the genome sequence into digitized code, we can send the digitized version of DNA code in the form of an electromagnetic waves with the speed of Light to any corner of the Earth then using a unique receiver at a distant location, we can re-create Life. Our ability to sequence the DNA code of all living forms including deadliest virus like the COVID-19 and then to send the entire sequence to any country in any part of the world to develop vaccine by-passing all international restrictions within second holds all kind of possibilities when it comes to developing new vaccines to treat various viral infections. We are moving towards a borderless world in which electrons and electromagnetic waves will carry digitized information across international borders to deliver sequences to experts anywhere in the world. The sequenced genomes, once converted to the digital code will be carried upon those waves and will be delivered to experts anywhere at the speed of light.

Today, we are technologically so advanced that COVID-19 virus was identified, its Genome was sequenced, and vaccines were developed against the virus within months. In future, when we have colonized Mars, we can beam the sequences of any plants and animals to our fellow humans on Mars within less than four and a half minutes. On the other hand, once humans colonized Mars, they can sequence the genomes of Martian Fossils or Microbes. If the life form on Mars is DNA-based and if we can obtain genome sequences and the Martian residents beam the sequence back to Earth, using the material available on Earth, we should be able to reconstruct the genome of the Martian life forms. The synthetic version of the Martian genomes could then be used to recreate Martian life for detailed studies without having to deal with the incredible logistics of bringing the sample back from other planets. We can re-construct the Martians life-form in a P4 spacesuit Lab that at is maximum containment Laboratory. Instead of risking them splashed down in the ocean or crash landing in the Amazon.

With this knowledge in hand, now we can attempt to answer the old questions. Questions like Who are we? Where have we all come from? What was it that made us this way? How this Universe began? Why is the Universe expanding at an accelerating speed? How is it likely to end? Are we alone in the entire Universe or there are other creatures who live in deep dark space of this vast Universe who may or may not look like us? Are we alone in the entire universe or are there other intelligent creatures and technologically advanced Alien, living in Alien Worlds? What is our place in the Universe? How common is life in the Universe? Are there UFO (Un-identifying Flying Objects)? Where should we look for intelligent life in the Universe? Why do we think that there is life in the Universe? Is there anyone listening to our radio signals? And what message Aliens could send us? If you look at the night sky, you must have wondered, of all the billions upon billions of points of lights in the sky, each point of light representing a star like our sun, why our own planet is thriving with life and why not others. If we were the only creatures in the entire universe, the vast empty space would be a vast wasteland. Would not that be a waste of vast space to have no one else besides us? What are our hopes and our dreams for the future of our exploration of space? We live our life in the future. If we have any future, then we must devote our future for the deep exploration of space. It is time to redefine the limits of our knowledge and it is time to redefine the limits of our technology. In short, it is time we prepare our next generations of Astronauts on a one-way trip for the deep exploration of space and ultimately colonization of outer worlds. Our aim should be to protect, preserve and spread human intelligence in every corner of the Universe.

How do we find out if we are alone? How do we search for life in Alien Worlds? Since the distances are so vast and life is so short, is human travel into deep space feasible? Before we begin to answer any these questions, let me share with you what astronomers have been doing for more than half a century.

Astronomer Dr. Frank Drake of West Virginia Observatory started searching the sky for any oncoming radio signals from Alien since 1961 under a program called SETI (Search for Extraterrestrial Intelligence). To this date, he has not received a single reproducible and verifiable radio signal. There has been an eerie silence in the space. Does that mean that there is no intelligence in the Universe who would like to communicate with us? The answer is certainly not. Since the distances among different solar systems are millions of light years apart, the probability of finding life is very small, but if we do not try, the chance of finding life is certainly zero. When Christopher Columbus left Spain in search of America; he sailed for almost 90 days before he found land. Suppose if we were to ask him after 30 days on the ship that what he had seen today? He would probably say water. What did you see yesterday? He would probably say water and the day before, he would again say water.

Even on the 89th day, he would say that he saw only water, but on the 90th day, he saw the land. He saw America. For over 60 years, we have been searching sky for any Alien radio signals and we have found none. Does that mean that there is no intelligent life in the Universe? We may have to look for another 60 years or may be longer or may be shorter. May be life is too precious; its occurrence is very rare, and we are alone in the entire Cosmos, and we must try to preserve this precious life, but we must find out if it is true. Most astronomers believe that we are not alone in the entire Universe. Nonetheless, yet we have not searched long enough and hard enough to know the answer for certainty. So, my answers to the above questions would be purely speculative, but they would be based on the scientific rationale.

In our solar system, we have one sun, eight planets and 140 moons revolving around our Sun; we call this organized collection of objects as our Solar System. In the Milky Way galaxy (our home galaxy) alone, which is 100,000 Light years across, there are about 400 billion solar systems like ours. There are about 100 billion galaxies in the visible part of the Universe. It is hard to imagine that there are no other living creatures on any of those billions upon billions of solar systems. Milky Way galaxy is not even the biggest galaxy in the known universe; it is an average galaxy. Our solar system is not the biggest solar system in the Milky Way galaxy; it is an average solar system. Compared to other solar systems, our sun is a middle age star. It has been burning its Hydrogen fuel for over four and a half billion years. It has already used up more than fifty percent of its Hydrogen fuel. Our Sun is composed of seventy-five percent of Hydrogen and twenty-four percent of Helium. The remaining one percent consists of other elements. There are billions of stars in our Milky Way Galaxy which are brighter than our sun and there are billions of stars dimmer than our sun. It is most logical to assume that on many older star systems, there would be abundant conscious, intelligent and technologically developed civilizations. Since they were formed before our solar system, we assume that they would be far more technologically advanced than our civilization.

The laws of physics and chemistry govern planet Earth also govern the entire Universe. Accordingly, if similar conditions exist as we have on Earth such as liquid water, Oxygen, and warm temperature, also exist elsewhere on any of the solar systems, could life have evolved with great ease as it did evolve on Earth. Although our Solar System was formed about four and a half billion years ago, microscopic life evolved on Earth within a billion year. Life did not waste much time evolving on Earth. Microfossils have been discovered in a 3.5-billion-year-old root of fossilized trees found in Pilbara Hill, in Australia. It appears that we are neither unique nor unusual, but we are the results of the four and a half billion years of biological evolution. In this vast firmament many solar systems exist like ours and some must have evolved before us and some after us; many Alien Worlds must be older than our own; their civilization could be much more advanced than ours. Human curiosity forces us to examine, if there are Alien Worlds or are we alone in the entire Universe. for thousands of years, we have asked ourselves simple questions like; why are we here? Why our Universe exists? How did Earth form at all? How and why life originated and populated our planet? Are we alone? Is there life out there? Who else is out there? As our knowledge expands, we are closer to answer these questions in the most scientific way. Our Sun is one of many stars among billions of stars in our Milky Way Galaxy and there are at least four hundred billion galaxies in the visible part of our Universe. Knowing that all stars have at least one Earth like planet, there are millions of habitable planets in our Milky Way Galaxy alone. There is bound to be life in few of those planets.

To answer the question, why are we here? We are here to explore the Universe. Without intelligent life, Universe was meaningless like wasteland; there was no purpose. The Universe was always there, it will always be there. But with intelligent life, Universe becomes purposeful. Our purpose is to protect, preserve and spread human intelligence in every corner of the Universe. Soon after the Big Bang about 13.72 billion years ago, the Universe began to cool, gravitational forces attracted, and the material began to condense forming island of galaxies in which was formed Star Systems like our Solar System. The revolving burning material condensed to form Sun and the cooler material condensed to form planets. Planets such as Mercury and Venus are too close to Sun are too hot to support life. Planets too far from Sun such as Mars, Jupiter, Saturn, Neptune, and Uranus are too cold to support life. Planet Earth is in the habitable Zone. It is neither too hot nor too cold. Life is evolved. The early hot Earth was bombarded with comets which brought water to Earth surface forming oceans. Seventy percent of Earth surface is covered with Water. Planet Earth is a Water World. In Summer, Water evaporates and in winter it condensed. Thunders and lightning storms cooled the planets even further. A million-lightnings strike Earth each day. At some remote corner of the Earth, lightning struck at a cloud of gases consisting of Ammonia, Carbon dioxide, Water near a Phosphorus rocks forming the RNA (Ribonucleic Acid) the first information molecule for creating life. Our early Earth was an RNA world. RNA can store information like DNA and catalyzed reactions like proteins. According to Darwin, life evolved, and nature selected. More complex life-giving molecules were evolved such as DNA (Deoxy Ribonucleic Acid) which store information, and Proteins which carry out body function, Carbohydrates provide energy, and Hormones to support life. These are all scientific facts.

Science means knowledge and knowledge is obtained by conducting experiments. It provides reproducible, verifiable results. For example, Water boils at one hundred degrees Centigrade and freezes at zero degree Centigrade. The first experiment we teach our students to conduct is to boil and freeze the water to see if all of them get the same reproducible results. Science is the cleverest form of detective work. There is no magic, no mysticism, and no miracle; it is all scientific facts and can be verified by anyone with some training.

Let me explain how our solar system was evolved, could such solar systems also have evolved in the Universe under similar conditions. Could life evolve under similar conditions? The answer to the origin of our solar system is beginning to emerge. It is now clearly understood how the journey of our solar system began and how much do we know the answers to some the following ancient questions we have asked ourselves since the dawn of human civilization:

What is Our Place in The Universe?

Long ago, in the far end of the Milky Way galaxy, on the third arm of the Orion Nebula, there was a massive explosion, a supernova exploded with a titanic force. When the massive Nebula used up most of its hydrogen energy, (Hydrogen fused to form Helium and generate sunlight with other rays). The Orion Nebula started burning its Helium gas, and then Helium is burned to Carbon, Carbon to Silicon. As it converted one element into another, it started cooling, the thermal forces pushed the Nebula to expand outside, and the gravitational forces attracted inside. Once it started cooling, the thermal forces gave in and the gravitational forces succeeded, the Nebula collapsed on itself exploding with a titanic force. At the instant of explosion, every element fused with every other element to form all the 94 elements found on Earth today (Of these 118 elements, 94 occur naturally on Earth. Six of these occur in extreme trace quantities: technetium, atomic number 43; promethium, number 61; astatine, number 85; francium, number 87; neptunium, number 93; and plutonium, number 94) that are described in the periodic table that include from the lightest element to the heaviest elements such as Uranium, Plutonium, Iron, Silver, Gold etc. In fact, all heavy elements found on Earth was the results of that super nova explosion about four and a half billion years ago.

The solar nebula produced prodigious amount of dust particles, gasses etc. These cloud of gas spin and swirl over billions of years crushing the Hydrogen nuclei forming Helium atoms releasing sub-atomic particles as sunlight. This process sets a thermonuclear reaction which crushed Hydrogen nuclei to fuse to burn igniting our Sun. The outermost gases and dirt formed rings around our Sun, which condensed over billions of years to form our Solar System which include the eight planets including our Earth. Every atom in our body, including the iron molecules in our blood were formed by the super nova explosion billions of years ago. In fact, every part of our body once was the part of the supernova. We are made of star stuff. If we live on Earth, we will consume everything that was made in the super nova.

Nothing lasts forever; we have a finite time on Earth. Hardly anyone lives past the age of 100 years. We will all be replaced by a new set of people within a century. When we die, our bodies decomposed turned to ashes; we become part of the star stuff once again. Over the past two million years since the human evolved, more than 20 billion people have come and gone, born, and died and became star stuff. Even the solar systems have a finite lifetime. During the next five billion years, when sun used up all its energy, it will also collapse and explode with titanic force and will become a black hole. The fire and the dust storm created

by the sun's explosion, will engulfed the entire solar system with its eight planets, 140 moons and billions of asteroids and comets revolving around the sun and once again our solar system will be turned to ashes of the star stuff. Even the universe has a finite time.

In 1920, Astronomer Edwin Hubble, observed that the Universe is expanding. All galaxies are flying away from each other. The farther galaxies are flying away even faster. When he calculated back to find the time of origin, he found that about 13.72 billion years ago, all the mass in the Universe was concentrated on one point. The Universe was a single mass of energy and then something happened, May be God said, let there be Light and there was Light. The Universe exploded with a Titanic force. Over eons, the energy cooled, the subatomic particles joined together by gravitational forces forming atoms. Further cooling results in the formation of molecules of elements and dust particles. The energy cooled and converted to mass. The gravitational forces attracted the matter further to form clusters; we call those clusters island universes, which condensed to form galaxies. Individual clusters of dust and gas storms, condensed to form sun and planets.

Our Milky Way Galaxy is about 150,000 to 200,000 light-years across, and about 2,000 light-years deep, and has 100 to 400 billion stars. It is an island of stars we call home. If you imagined it as a disk with spiral arms emanating from the center, our sun is about a third of the way from the center to the visible edge. Our spiral arm is formally called the Orion-Cygnus Arm. It's also known simply as the Orion Arm. Our sun and its planets reside about 26,000 light-years from the center of the galaxy, on the inner edge of the Orion-Cygnus Arm. Orion Nebula is an enormous cloud of gas and dust, one of the many in our Milky Way Galaxy. It lies roughly 1,300 light-years from Earth. It is sometimes called the Orion Nebula Star Cluster.

Over the years, astronomers have observed, although the Universe is expanding, the overall rate of expansion will slow down as the existence of dark matter is confirmed. Most astronomers believe that the Universe is made of ninety- percent invisible matter that could not be detected by spectroscopic techniques, they call it dark matter. If there is not enough dark matter in the Universe, a time will come when the rate of expansion will slow down and come to a halt. The expansion will run in reverse direction, contracting the Universe. It will start collapsing upon itself. In about 80 to 100 billion years from now, the Universe will collapse on itself with a titanic force. All material will rush towards its center. Time will move in the reverse direction. The Universe will end up as a Big Crunch. What happened next? Will the Universe expand once again and collapse once again? Is Universe alternating between Big Bang and Big Crunch?

Radioactive dating technique shows that our Earth was formed about 4.6 billion years ago. The emergence of Life on Earth is no less than a miracle. If you examine the origin of life on Earth, it becomes quite clear that once the primitive Life emerged when the mixture of Carbon, Hydrogen, Ammonia is exposed to thunder and lightning. Obeying the laws of physics and chemistry, the molecules of Carbon, Hydrogen, Ammonia, Phosphorous etc. react to inform information molecules called nucleotides. It is the nucleotides that organized themselves to become alive. As soon as Life evolved, Darwin Laws of Natural Selection takes over. Environmental conditions and Natural Selection forced a unicellular life to evolve to multicellular life form leading plants and animals and leading to intelligent life on Earth. In almost three billion years, Life evolved from a primitive single cell creature to more advanced and technologically superior Life forms like us. If we examine the fossil records of the evolution of Life on Earth, the oldest micro fossilized bacteria were found in Western Australia in a 3.5-billion-year-old rock. Life on Earth appeared soon after it cooled from the super nova explosion. Although it had taken over a billion years for life to evolve, in geologic time, Life appeared very quickly on Earth. If similar life support conditions such as moderate temperature, Methane, liquid water and oxygen exist elsewhere in the Universe; life could emerge as quickly as it did on Earth.

There is every reason to believe that life exists in every corner of this vast universe. Universe must be teeming with life. You might ask if there are Alien civilizations exist somewhere in the Universe why we don't see them. The answer is that the distances between star systems are so vast, one lifetime of 100 years is not enough for the journey for creatures like us to travel those long distances. For example, the nearest star to our sun is called Alpha Centauri (Proxima Centauri). It is a binary star system. There are two suns. Since we have one star, the sun, and we also have eight planets that make our solar system, let us suppose Alpha Centauri having two stars should have 18 planets. Since we have only one habitable planet Earth, we assume that Alpha Centauri has two Earth like planets. It is also possible that Alpha Centauri planetary system did not allow Earth like planet to form and life to evolve. If we go in search for the Earth like planets in Alpha Centauri, it will take an extremely long time.

How long would it take us to visit Alpha Centauri and return to Earth? It would take too long. It is too far. You might ask how far is too far. Let me give you some idea of the distances involved. Light travels with a speed of 186,000 miles per second or 300,000 Kilometer per second. In one-year, light particles called photons cover six trillion miles. We call this distance a light-year. We cannot travel faster than the speed of light. This is called the Einstein Speed limit. Einstein speed limit in space places a restriction on space travel that nothing can travel faster than the speed of light. Light takes about 8 minutes to reach Earth from Sun. Our Sun is about 93 million miles from here. So, in 8 light minutes, it covers 93 million miles. Alpha Centauri is located about four and a half light years from Earth. It is about 26 trillion miles from Earth. If you take a spaceship and travel with the speed of light, your clock will tic far too slowly. If you go to Alpha Centauri and return without stopping there, according to your clock, you were in the spaceship for only nine years. The clock on Earth moves much faster since Earth rotates much slower on its own axis. Earth has a circumference of 25,000 miles. Earth rotates on its own axis and completes on circle in 24 hours to complete a full day. In an hour, it rotates about 1020 mile.

According to your calendar, you were in the spaceship for only 9 years, but on Earth 160 years would have passed. No body live that long to greet you upon your return journey. One hundred years human life span is not long enough for a journey to Alien Lands. I am afraid that those people who came to see you off would all have been dead and gone. We have a speed limit in space, and we have an age limit of humans. How should we attempt to communicate with Alien beings if they exist in the far corner of the universe? We have sent four unmanned spacecraft in four different directions. They are Voyager I and Voyager II, and Pioneer 10 and Pioneer 11. They have traveled over ten billion miles and have already left our Solar System. How far could they go? If there are any Alien civilizations within a hundred Light year, residents of that region of space will need to be patient if they want to see Pioneer 10. To see another star system, NASA expects the spacecraft to travel for another 2 million years or to travel 68 light-years of

space to reach the Aldebaran star system. Aldebaran is one of the 15 brightest stars, with an apparent visual magnitude of 0.85. Its diameter is 44 times that of the Sun. It is accompanied by a very faint (13th magnitude) Red companion star. Aldebaran lies 65 light-years from Earth. Aldebaran is 6.605 billion years old. This makes Aldebaran far from the oldest star, yet it is still older than our own sun.

As I said above Earth rotates on its axis and completes one circle in 24 hours. Our Earth rotates around Sun and completes one circle in 365 days. We call this journey of Earth a year. Our sun also rotates on its own axis every 27 days. Our Solar System is also revolving around our Milky Way galaxy every 250 million years. Our four unmanned spacecraft would not have enough energy to leave the gravitational attraction of the Milky Way galaxy and leave for Andromeda galaxy about two and a half million light years away. Since Milky Way galaxy is spiral shaped and it alone harbors over 400 billion Solar Systems, if there exists a single technologically advanced society, we might be able to communicate with them.

How Common is Life in The Universe?

In 1953, Stanley Miller, a student of Nobel Laureate Harold Urey, of the University of Chicago conducted an experiment in the laboratory to show if he could make chemical building blocks that make life. He assembled in a flask commonly occurring elements on primitive Earth such as carbon dioxide, methane, ammonia, water, and spark electricity to mimic thunder and lightning. Within a week, he observed that the solution in the flask became dark. Analysis of the solution showed that he had made organic molecules such as amino acids that are the building blocks of all life forms on Earth. NASA also demonstrated that basic components of the life giving amino acids are also formed in the upper atmosphere. Amino acids are also made from two other organic molecules called formaldehyde and hydrogen cyanide. Spectroscopic analysis of the upper atmosphere showed the presence of formaldehyde and hydrogen cyanide in the vicinity of many distant star systems. Although these chemicals are toxic individually, but in combinations they are essential components for the evolution of life. They also exist in the vicinity of the far away stars billions of miles from Earth. We have learned that the chemistry near stars is the same as the chemistry on Earth. Organic molecules are precursors to life and are very common in the Universe and, therefore, life must also be common in the Universe. The distances between star systems are so far that physical travel to distant stars systems is impossible currently. We could still communicate with the Alien civilization by radio communication if they are at least as advanced as we are. We are interested not just in life, but intelligent life like ours, technologically advance life, and may be more advanced than ours. An advanced life that could generate radio signals as we do on Earth. If we find radio signals like the one that we make anywhere in the Universe, we will probably find life in that part of the outer world. Few years ago, we did receive such false radio signals and were confirmed by several observatories around the world. There was a lot of excitement in the SETI (Search for Extra-Terrestrial Intelligence) community. Soon we found out that it was a hoax; it was a man-made satellite called Solar Research satellite created those signals. We launched that satellite several years earlier to study Sun's spots. It is orbiting around a million miles from Earth.

Are There Ufos (Unidentified Flying Objects) in the Universe?

From the above explanation, it becomes clear that interstellar travel is impossible currently. In future, time travel could become possible if we were to discover wormhole in our galaxy and learn how to survive time travel in wormhole. For that reason, we say that so far, no aliens visited Earth. If someone claims that he has seen Un-Identifying Flying Objects (UFO), you will immediately say that what they saw was the man-made objects thrown as debris in space. Suppose this person is right, you might wonder. How can you carry all the necessity of life support systems with you on such a long journey? Carrying enormous amounts of food, water, oxygen for billions of miles long journey is impossible and for what reason, to meet with us, to greet us, or to carry us to the Alien Worlds. Why should they take such hazardous journey and for what purpose? If aliens are so technologically advanced, they will send an unmanned space probe to Earth to collect a single human hair from an eyelash, decipher the entire genetic code and create clones of humans in their worlds.

Those people who claim to have seen UFO, what they are saying is that these objects exist inside their heads not outside. It gives us a window to investigate their miserable life. Why no UFO visited India, but they are visiting America. The answer is simple no one will pay to buy such stories and UFO products because no one believes in this hoax. Americans buy the UFO products, such as facemasks, literatures, films etc. They have developed an industry to sell these products and they make money. UFO enthusiasts have a kind of festival in a place called Roswell in the state of New Mexico in America. They believed that a Flying Saucer crashed in December 1947 near Roswell and United State Air Force is hiding the debris from the public to avoid panic. This was a nonsense story and was perpetrated by media such as newspaper and TV to attract public attention. The fact is as follows: During 40s, the cold war between Russia and America was heating up. America had detonated a nuclear bomb and Russia responded accordingly. Americans were working on developing Hydrogen bomb and the American government was anxious to know if Russians were also developing Hydrogen bomb. The American government has given a grant to the New York University research group to launch a weather balloon to detect shock waves generated by the Hydrogen Bomb explosion and record any radioactive cloud generated by the secret Hydrogen bomb blasts. These balloons eventually collapsed and crashed and were stored in hangers in Roswell warehouses. Not to announce to the world and particularly to keep Russians in the dark, the project was kept secret. Another similar research grant was made available by the American government to the University of Ohio under the name of Project Blue Book to keep monitoring Russian space program. UFO enthusiasts believe that under the name of Project Blue Book the American government has been gathering evidence about the UFOs and hiding in a military warehouse. Two university investigation teams reviewed the evidence and found them to be non-UFO. If someone still insists that there are UFOs, I suggest that you ask the following questions: Why do those who claim to see UFOs see them in the sky only, why don't they land on ground or water? Why don't they leave any evidence? Their home address or their telephone numbers would be good. We are looking for any evidence. When we climbed on Mount Everest, we left a lot of trash behind, such as water bottle, food cans, eyeglasses etc. When we went to the bottom of the deepest ocean, we left similar trash. The ocean floor is littered with our trash. Even when we went to space, we discarded millions of pieces of junk in space. When we landed men on the moon, we left plenty of trash on its surface. Why don't Alien leave any trash if they have visited us, not even a plastic bottle or a food can? Why UFOs are visiting us during the last 50 years, why not before? The Earth was formed about four and a half billion years ago, why UFOs are visiting us now? Have these people seen something in the sky? I believe that they have.

What they saw is probably debris of micrometeorites showering Earth. Planet Earth is being bombarded with millions of micro-comets and micro-asteroids all the time. As they entered our atmosphere, due to air friction, they catch fire and burned. No traces of them reach Earth. On the other hand, if big chunk entered our atmosphere, small part of asteroids is recovered on Earth, mostly from the icy surface of Alaska.

Most scientists are convinced that the Universe is teeming with life, but they are also convinced that no extraterrestrial creature from Alien Land had ever visited us. Our search for other star system like solar systems in our Milky Way galaxy began in earnest when we launched the Hubble Space Telescope about 30 years ago. Hubble Space Telescope was launched on April 24, 1990, aboard the Space Shuttle Discovery. Hubble is currently located about 340 miles (547 km) above Earth's surface, where it completes 15 orbits per day, approximately one every 95 minutes. Hubble will continue to build on its 31-year legacy, broadening our horizons with its view of the universe.

Drs. Jeff Marcy and Paul Butler at the Berkeley campus of the University of California have confirmed Swiss astronomer's discovery of Earth like planets and identified several star systems with Jupiter size planets. Jupiter is the largest planet in our solar system. It is so large that a thousand Earth like planets can be accommodated inside Jupiter. It is failed sun. So far astronomers have discovered more than 500-star systems with more than a thousand Earth like exoplanets in them. Are there any living creatures on any of them? We can answer the question with a question. Are there Earth-like planets with life support systems in any of those solar systems? Astronomers have been searching for Earth like planets that have life support systems, most importantly liquid water. To predict Earth like planet, we need Earth like conditions, plenty of Water, Oxygen, and warm temperature. Seventy- percent surface of our planet is covered with liquid water. Today, Earth has 20 percent Oxygen and almost 80 percent Nitrogen and trace amount of Carbon dioxide, Ozone etc. Primitive Earth has no Oxygen. Oxygen began to accumulate in the atmosphere after the origin of life on Earth. Plants and trees must have been the first living creature to evolve at surface of the Earth because plants produce oxygen. Without oxygen, our kind of life cannot survive. Every molecule of the oxygen, we inhale today, was produced by plants. Oxygen is very reactive. If primitive Earth has any oxygen left, it would have reacted with metals such as iron to form rust, the iron oxide. Comets brought every drop of water on the early Earth by massive bombardment during the formation of our planet.

Suppose some Earth like planets in nearby solar systems do evolve life. A primitive life form would be no use to us. The continent of Australia was separated over 50 million years ago, Marsupials thrived, but never developed human like consciousness or intelligent or technologically developed society. During Jurassic period, Dinosaurs (terrible lizard) ruled our planet Earth for over 150 million years. They never developed intelligent. They all perished when a comet struck Earth about 65 million years ago generating thunder, lightning, shock wave, dust, mud rain which blocked sunlight for years. Most creatures were trapped in that mud rains and perished. The dust, smoke and debris cut out sunlight and planet Earth plunged into darkness for almost three years. In the absence of sunlight, oceans froze, and all living creatures perished. When the dust settled down, sun began to shine again, plants began to grow and thrive again. Evolution moved in a different direction; it was the mammals that moved up on the evolutionary ladder this time.

If the comet had come 20 minutes late or 20 minutes early and missed our planet, we would not have been evolved and would not have been here at all. May be Dinosaurs would still rule Earth. Primitive human bone fossil was discovered in a three-million-year-old rocks found in the Afar Valley in Ethiopia. We found the fossils of many extinct creatures, but no human bones were ever found near Dinosaur's fossil. Our primitive ancestor was hiding from flesh eating Dinosaurs. It was after the death of Dinosaurs that our ancestors, Mammals, came out of hiding and were evolved. Although there were living creatures roaming around the world, but none had the intelligence or ability to create technology and to communicate with radio signals with Aliens if they were to try to communicate with us even hundred years ago. We need technically advanced society to communicate with the inhabitants of the Alien Land.

Is There Alien Life in The Universe? Some Say Yes and Others Say No. Then Came the Real Surprise

On June 25, 2021, the US national intelligence community released a long-awaited report on UFOs. In recent years, several poor-quality videos taken by Navy pilots showing mysterious objects some of which appeared to be moving at astonishing speeds and with motion unlike any known by terrestrial aircrafts. As I said above, the speed of Light is 186,000 miles per second, and nothing can travel faster than the speed of Light. These vehicles move in a way that defy the laws of physics. So, to do space travels faster than the speed of Light; you really need Wrap drive and there is no such thing as Wrap drive it is a fiction not a fact. Science is a candle in the darkness. After examining the footage and records of objects in the skies, the scientific answer is that we don't know exactly what's they are. This was a real object. Millions saw it on the TV broadcast and on their computers. Is this a robotic spacecraft trying to establish the first contact with humans? Where is the mother ship? Is this robotic daughter ship communicating with the mother ship? Could we directly contact the mother ship? Now, we have learned to convert Analog language of Biology to the Digital language of computer, we may be able to send our genetic code with the speed of light. After all we invited Aliens.

About 70 years ago, we sent and invitation to Alien civilization to come and visit us. On March 2, 1972, NASA launched its Pioneer 10 spacecraft, which would become the first craft to travel through the asteroid belt. About a year later, Pioneer 11 took flight. Pioneer 10 and Pioneer 11 carry a plaque that features a design engraved into a gold-anodized aluminum plate attached to the spacecraft's antenna support struts to help shield it from erosion by interstellar dust. With our own images craved on the Plaque, the plaque also provides our home address. Our location in the Milky Way galaxy which is on the third arm of the spiral galaxy. Among billion of stars, our location is on an average star, the Sun, around which revolve eight planets and 140 moons with millions of asteroids and comets forming an average Solar System. Our home address is Earth the third planet from the Sun. Both Pioneer spacecrafts have left our Solar System. They are about 10 billion miles from Earth. Have the spacecrafts been retrieved by a technically advanced Alien civilization? Have they sent an emissary to meet with us? Are they trying to contact us? How should we respond? Has anyone in the SETI community seen this object? Are they still waiting for radio signals? Some say that we have not found any crash site with alien DNA for analysis. What if the object sighted carries no living creature? Suppose it is a robotic spacecraft collecting data on human activities? We all know that the nearest star is Alpha-Century which is located four and a half Light Year away. Currently, neither we nor UFO can travel that long distance. Extraordinary claims require extraordinary evidence. This is a

gatekeeper rule of sorts. So, you think in all the universe, among billions and billions of galaxies each with billion and billions of stars that including untold numbers of planets, we humans are the only form of intelligent life? According to Carl Sagan if it is true as he said, "What a waste of space." Someday, Interstellar space travel is possible, and we have not achieved it yet and what if the Aliens have achieved the impossible?

We always assumed that Aliens have hostile intention. Because of the vast distances, we have no threat from each other. Let us suppose, they are visiting us with friendly intention. Let us share our knowledge, our hopes, and our dreams of Space travel with them. We have lot of information to share with each other. Since they have conquered space travel, we have the following questions to ask them:

Is There Life on Our Solar Systems Beside Us?

Although our solar system has eight planets and 140 moons, only Mars in its history had life supporting conditions such as liquid water, most critical condition for life to evolve. Among the 140 moons, two moons could support primitive life. There are 16 moons of Jupiter; two of them could support life and they are Europa and Titan. After traveling more than half a billion miles, our unmanned spacecraft Galileo has been orbiting Jupiter since December 7, 1995. Only on May 20 of the year, 2000, spacecraft Galileo came close to 503 miles of Ganymede the largest moon of the Jupiter. The photographs taken by Galileo showed that even Ganymede has Earth like salty ocean hidden about 120 miles beneath the huge moon's ice-and-frost-covered surface? Ganymede has a fractured and cracked surface highly suggestive of a watery or slushy ice that may have surfaced through the fractured crust creating geological patterns like those found on the surface of Europa.

Microscopic life could exist on any of those four heavenly bodies, but intelligent life with technically advanced society like ours could not have evolved. Microscopic life could survive under harsh conditions and extremophiles are known to exist on Earth under extremely harsh conditions. Mars, the red planet, is our closest neighbor. It is about 120 million miles from Earth. In 1976, we landed an unmanned Viking spacecraft on the surface of Mars. We found no liquid water. The spectrometer detected no life-giving gases. It is a barren frozen red desert. Even in summertime, the surface temperature remains several degrees below zero. In 1997, we landed an unmanned spacecraft called Pathfinder that crawled on the surface of Mars analyzing samples and sending data back to Earth. In its distant history, Mars must have liquid water flowing in rivers. The dry river grooves on its surface indicate that once Mars was a watery world. Somehow the atmosphere disappeared. Probably lack of enough radioactive material as we have under Earth releasing energy, heating earth crust that moves plates tectonic causing magnetic field and controlling weather system.

Mars is a frigid, frozen, barren desert. The atmosphere on Mars is less than one percent what we have on Earth. If you pour a glass of water on the surface of Mars, it will evaporate before hitting the surface. Mars is half the size of Earth. Because of the absence of atmosphere, Mars has no surface water and most of the water is either evaporated in the upper space in its history or has gone underground as permafrost. Planet Mars also has volcano. Underground valleys near volcano could melt ice where microscopic life form could have evolved. In December 1984, a U.S. meteorite-hunting expedition, discovered in Antarctica, a meteorite, a rock that fell to Earth from space (ALH 84001), a team of American scientists announced the analysis of a potato size meteorite found to contain remnant of microscopic life form. There is no doubt that meteorite must have come from Mars. Two pieces of evidence confirms its origin. First, all rocks in Antarctica are covered under a mile thick sheet of ice. Any rock found on its surface must have come from somewhere as meteorite. Second, the unmanned spacecraft Pathfinder analyzed soil sample on surface of Mars that matches with the composition of the meteorite sample found on Antarctica. What is highly controversial is that the microscopic examination of the structure of the rocks could not determine with certainty if the structures are organic or inorganic in origin? Whatever the scientific community decides, there is not technologically advanced or intelligent society that supposedly builds canals on Mars. We have seen Martians in the films and have read them in the storybooks and even heard of the Martians invasion of Earth on the radio. One thing is certain, Martians are everywhere except on Mars.

Experiments prompted by a 2008 surprise from NASA's Phoenix Mars Lander suggest that soil examined by NASA's Viking Mars landers in 1976 may have contained carbon-based chemical building blocks of life. A key scientific objective of the mission is to determine how the climate of Mars has changed over time and where water resides on Mars today. In 2008, NASA (National Aeronautic Space Administration) landed the robotic rover Opportunity on the surface of Mars to study the Victoria and the Endeavour Craters. NASA has sent five robotic vehicles, called rovers, to Mars. The names of the five rovers are: Sojourner, Spirit and Opportunity, Curiosity, and Perseverance. Despite its frigid desert conditions, NASA plans to colonize Mars before this century is over. Within a hundred year, we will become the first Martians. Survival of human civilization is essential anywhere outside Earth. If nuclear wars, environmental collapse, or asteroids destroy Earth, humans could still survive on other worlds.

In search of life on the remaining planets and moons on our solar system, during the last two decades, we have sent four spacecraft two Voyagers and two Pioneers. By now all four have left our solar system. The two were Voyager spacecraft Voyager I and Voyager II that flew by the two moons of Jupiter and the other two were Pioneer 10 and Pioneer 11. The reconnaissance photographs taken by these spacecrafts showed that the surface of Europa, one of the moons of Jupiter, is covered with thick sheet of ice. During its summer thaw, the outer water layer showed cracks on its smooth surface. Life could have evolved under such harsh conditions underneath the frozen ocean. We cannot prove the existence of microscopic life on Europa until we send an unmanned spacecraft on its surface and drill into ice layer to detect any kind of life form. The moon of Saturn called Titan is the most likely moon to be studied extensively in this century. It has clouds of gases like those on Earth. Underneath those clouds, there could be ocean of water or ammonia gas or methane. Life as we know could not evolve in the presence of ammonia or methane gas. The unmanned spacecraft Cassini-Huygens arrived at Saturn on 1 July 2004 and explored the ringed Saturnian system in detail over an initial four-year period. It had a European space probe Huygens that launched from the spacecraft Cassini to land on the surface of Titan. Huygens landed on Titan on 14 January 2005. The probe showed as predicted ocean of Methane and pebbles, which may be made of hydrocarbon-coated water ice, with temperature below 179.3 Centigrade. Under these conditions Titan cannot support life. During this period, the spacecraft discovered two new moons, Daphnis, and Anthe. It had also uncovered much valuable data about Titan, including the first radar images of the moon's surface

taken during its Oct. 27, 2004, flyby. Scientists concluded that on Titan, the feeble sunlight allows only about one centimeter of evaporation per year (versus one meter of water on Earth), but the atmosphere can hold the equivalent of about 10 m (30 ft) of liquid before rain forms vs. only a few centimeters on Earth. So, the weather on Titan is expected to feature torrential downpours causing flash floods, interspersed by decades or centuries of drought. Titan was assumed to be so much like Earth. Now, we know that Titan is unsuitable for human habitation. Titan was a great disappointment.

In our entire Solar System beside Earth not a single Planet nor their Moons are suitable for human settlement. If we want to colonize Mars, we must terraform its weather to build human settlement on Mars. In search of Earth like planet, we must search for an exoplanet beyond our Solar System. In search for a second home for humanity, we must develop new technologies to prepare an army of Astronauts, who are willing to travel into deep space on a one-way trip to an unknown destination who are free from pain and suffering, who are free from diseases and who live beyond one hundred years.

Our four spacecrafts, two Voyagers and two Pioneers, have flown by all our eight planets and 140 moons and have left our solar system in four different directions. These spacecrafts have detected no sign of intelligent life on any of those eight planets and 140 moons. With one hundred percent certainty we could say that in our Solar System, there is no intelligent life on any of these planets or their moons except us on Earth.

Beyond our solar system, we have billions upon billions of chunks of rocks, meteorites, and comets revolving around our solar systems called Kuiper belt. Beyond Kuiper belt, we must travel about 26 trillion miles or about 4 light years, to come across a double star system called Alpha Centauri. There are two solar systems joined together forming a binary star system. Will the gravitational push and pull of the two suns allow Earth like planets to form and life to evolve is not certain? Beyond Alpha Centauri lies an island of cloud and dust called Oort clouds. This massive collection of dust and cloud is a nursery of stars where stars are born and die. If we continue to fly with the speed of light for almost 8 years, we come across Bernard star. This is another solar system. Currently, there are no plans to send any unmanned spacecraft to Bernard star. In 2007, astronomers discovered a huge almost Jupiter mass size exoplanet orbiting nearby star Epsilon Eridian, and it is about ten and a half light years away from us. Beyond Epsilon Eridian star, we must fly for almost 100 years to come across a huge dust of cloud system called Vega system about a 100-light year from here. The astronomer Carl Sagan in his science fiction novel, Contact, had imagined that intelligent aliens have reside on Vega, have sent us a map to construct a machine, which could allow us to find the nearest wormhole to travel with the speed of light to meet the aliens. This is purely fiction. May be, we must look for life beyond Vega?

Where Should We Look for Intelligent Life in The Universe?
As I said above, water is essential ingredient for life. Three elements are plenty in the Cosmos and they are Hydrogen, Helium and Oxygen. At the time of Big Bang, Cosmos was filled with Hydrogen. As Cosmos cooled, the plasma cooled to form electrons, protons, and neutrons. They further cooled to form atoms and molecules. The gravitational force attracts the cooling matters to form islands of clusters called galaxies. There are 100 billion galaxies in the known universe. In galaxies, clouds of dust and gases spin to ignite fire that became stars like our sun. Each galaxy has at least 400 billion stars. Dust and clouds around stars condensed to form planets. Many a sun is born and many of them died. A dying sun expands as it cools. The expanding dust cools further and falls on itself setting up a supernova explosion. At the instant of explosion, lighter hydrogen atoms fused to form heavier elements. Life giving elements such as carbon, Nitrogen, Sulfur, and Phosphorus are formed in abundance. Our spectroscopic techniques have shown that precursor of life-giving molecule such as formaldehyde and hydrogen cyanide has been detected in the upper space. Helium is not reactive, but Hydrogen and Oxygen are, and they combine to form water. Comets are muddy snowballs filled with frozen water. Billions of comets in our Solar System alone are filled with ice. Unfortunately, this is all frozen water. We need liquid water for the life to start. As Darwin had said in his book, The Origin of Species, life must have begun in a warm little pond. Liquid water requires warmth. We must search for intelligence life in the Universe in the vicinity of warm planets.

Why Do We Think There is Life in The Universe?
As I said above, our solar system is 4.6 billion years old. Our sun is about 93 million miles (8 light minutes) from Earth. The next nearest solar system is Alpha Centauri that is about 26 trillion miles (4 and a half light year) from Earth. Barnard star is about 8 light years from Earth. There are about 400 billion solar systems in our Milky Way Galaxy alone, which is 100,000 light year long. Many of solar systems are much older than our solar system. They were formed 10 to 20 billion years ago. So far more than 4000 Earth like planets, exoplanets, have been discovered in the nearby solar systems. Starting life at the suitable planets is a quick process in geological term. On Earth, we have discovered microfossil in a 3.5-billion-year-old Pilbara rock in Australia. If technical civilization were to evolve on any of those solar systems, they would be far more senior and far more technically advanced than our own civilization. Humans were evolved on Earth in about 3 million years. Within this time, we become technically so advanced that we landed men on the moon and brought them back safely. If technically advanced civilization were evolved on nearby solar systems, they have not visited Earth so far. There is something called the Fermi Paradox. (Enrico Fermi was the Italian Physicist who participated in constructing the first nuclear bomb. He asked this question. Where are aliens?) Do Aliens exist? Have Aliens spread and colonized the Universe or is there any form of intelligent life existing in the Universe? How do we find out answer to this eternal question? There are two possible ways to deal with this question. Because of the vast distances existing among solar systems having Earth like planets, we can search for these answers by traveling into deep space by either building better machines or by building better humans. We are working on both projects by building better machines and by prolonging human life. The existing spacecraft flies with the speed of 17,000 miles per hour, comparing the speed with other vehicles on Earth, the speed seems quite fast. For space travel, it is very poor. For example, with this speed, it will take us 75,000 years to get to our next-door solar system, Alpha Centauri. You might say, we have been broadcasting Radio and TV programs for more than 100 years. Their signals traveled with the speed of light, the Radio and TV signals have been traveled for almost 100 light years. We have been sending these signals in every direction in a 100 light year radius. Several Solar Systems exist within 100 light years distance. Shouldn't we wait for a response from Alien? Some say, we have sent two Voyagers and two Pioneers spacecraft in deep space.

On March 2, 1972, NASA launched its Pioneer 10 spacecraft, which would become the first craft to travel through the asteroid belt. About a year later, Pioneer 11 took flight. And in 1977, NASA's Voyager 2 spacecraft launched, with Voyager 1 following behind a few weeks later. They have been traveling for the past 50 years and have traveled more than 10 billion miles from Earth and left our solar system long ago. Shouldn't we wait to see if these spacecrafts were picked up by Extraterrestrial (ET) signals who would probably send a response to their existence? While some say let us wait and other say how long. Here is the scenario. Suppose Spain had decided not to send Columbus to discover america. Look Columbus, they say that the cost of sending three ships and hundreds of men on a 90-journey to no specific destination is impossible to approve. The cost is very high. Instead, they say we make two suggestions. Why don't you write messages and put in glass bottles and release in the shore and then you pray? One day, your prayer may be answered and one of the bottles may float to America, picked by a native American who will send his response in another bottle and send it back to you.

At some future time, the bottle will return to Spain. This will save time and money. The other suggestion they say would say to Columbus why don't you wait for 500 years; we will discover the super jet and will fly you across the Atlantic Ocean in 5 hours. Our spacecraft two Voyagers and two Pioneers were similar bottles with messages sent into deep space for Aliens to find and respond. Now, do we sit and pray or do something. Suppose ET picked up one of our spacecraft five billion years from now. By that time, our Sun would have used up all its energy. It would expand to sallow planet Mercury, Venus and Earth and we would all be dead and gone and our solar system would be turned our planets to ashes. They would learn about our existence too late. The map we have sent with the Pioneer 10 would provide directions to our Blue Planet. By that time, our Blue Planet would have turn to ashes. Unmanned spacecraft can perform some function, but they cannot replace humans.

For that reason, most astronomers think that we, humans, would have to travel into deep space on a one-way trip. The reason is simple. Humans are self-correcting, self-adjusting living machines. We are also self-destructive. What if we go on a nuclear war and blow our planet away? Since we are planning to colonize planet Mars during this century, imagine our future generations on Mars read in their newspaper one fine morning about the beginning of nuclear war and end of our species on Earth. Human civilization could still survive in the Universe. Geological record of our planet shows a mass extinction of living species every 20 million years. If we don't blow each other away by nuclear war, what if Earth is struck by a comet as it did 65 million years ago that wiped out Dinosaurs, the mighty beast that ruled Earth for almost 150 million years. To preserve human civilization, we must colonize other planets, other solar systems in our galaxy. We may have to send a future generation of astronauts on a one-way trip into deep space to spread human intelligence in every corner of the Universe. Our spaceship Freedom is the prototype of the future spaceships to come. There is no air above 33 miles. Spaceship Freedom will teach us how to survive above 33 miles in a vacuum. If astronauts could learn to survive in spaceship Freedom, grow their own food, recycle their own environment, and regenerate their own water then they could survive in any part of the cosmos for any length of time.

Is There Anyone Listening to Our Radio-Signals Beyond Our Galaxy?

As I said above, we have been listening for an alien signal for the last 50 years and we have received none. There has been a eerie silence. Suppose within ten years, we receive the first signal, and this signal is real, and this is reproduced at the same frequency and is verified and confirmed by every listening station in every country of the world. What do we do next? Shall we respond and who will respond? What shall we say? Are they good or bad aliens? Do we provide our home address by giving the road map or the space map? Some say that it is a bad idea to give our home address to strange aliens. They may be unfriendly. Other says, if they are located about a 100-light year from us, what difference does it make weather we give our address or not by the time their message arrives, 200 light years would have passed on Earth.

None of us will be here even to receive their response. Many generations would have been dead and gone. They say give aliens our home address by giving the celestial coordinates for our location. This is how they could find us. We say look for our home galaxy Milky Way. It is a spiral shaped galaxy spinning on its own axis. It is easier to find because we have a vast empty space around us. Our next-door neighbor is the Andromeda galaxy that is located about two million light years from our galaxy. Once you get to Milky Way galaxy, there is a loop on the outer third arm called Orion Arm. Once you get there, you will find us on a middle-aged star system called sun. Around the Sun are revolving eight planets and 140 moons. We are the 3rd planets from sun and our planet is called Earth. It is the only blue planet in the entire solar system. You cannot go wrong, just follow our directions. The exact distance from the sun to our blue planet, Earth, is 93 million miles. There is no other planet close to us. By the way, we have just one moon. It is located at about a quarter million mile from Earth. Don't stop at the moon because there is no water or air on the surface of the moon. Only Earth has water and an atmosphere to support life system. Would they really follow our directions or stop on the way to some more interesting planet? It is unlikely that alien could travel to our solar system when they could find many more interesting star systems in their neighborhood. They could probably send us a message by radio signals.

What Message Alien Could Send Us?

As I said above that the astronomer Carl Sagan had imagined in his novel "Contact" that the Aliens would send us instructions to build a spacecraft to take us to the nearest wormhole that will make time travel a reality and will take us instantly into their neighborhood. What language will we use to communicate? What do we say? Since 1961, Frank Drake of SETI (Search of Extra-terrestrial Intelligence Institute) is waiting to receive radio-signals. Although 60 years have passed, he has not received a single reproducible signal so far. It may be that there is no technologically developed Life within the 60 Light Year distance to respond to any radio-signals. Moreover, we have been sending Radio and TV signals for the past 100 years in every direction. We have not received any reply. It tells us that there is no technologically developed intelligent life on any Star System within a hundred light years. It also tells us how rare life is in the Cosmos and that we must prepare for a long journey beyond 100 light years. We must learn to survive for centuries on our spacecrafts. We must learn to grow food, produce Oxygen, and recycle water for endless journey. Comets are water world. We must learn to capture comets and attach them to our spacecraft as a constant source of water and Oxygen for us and Hydrogen a fuel for a long journey. The SETI community has drawn guidelines for the community. Through United Nations, we all will send a single message. This is a Gentleman Agreement not binding to any nations or groups. Imagine a situation when we receive the very first message from the alien world. There are thousands of telescopes owned by governments and private individuals. Who could stop anyone if someone decides to send

his own private message? We must do a lot of homework draw guidelines to resolve these issues.

On February 24, 1987, a catastrophic event occurred in the Magellanic cloud about 163,000 light years from Earth. A supernova exploded with a Titanic force. The explosion was first recorded by Stephen Sheppard of the Toronto Observatory in Canada. Within three hours, every observatory on Earth confirmed this event. You might wonder why this event so far from Earth concerned us. The reason is that our Solar System is going through the same path. It is becoming a Red Giant and then will explode as Super Nova.

Super Nova is the result of a violent death of a star. A star explodes as a super nova, when it used up most of its energy and collapsed upon itself causing the tremendous explosion and releasing colossal amount of energy. The released energy fused the Hydrogen atoms to form all 120 elements that we find on Earth and all living and nonliving material including the element Iron in our blood to the Calcium in our bones. All these elements are formed from the explosion of dying stars.

Is Extinction Our Ultimate Fate?

Mother Nature may say Boys and Girls I have a bad new for the humanity. Your fate is extinction when the Earth dies you all will die with it. You can pray all you want, but when Super Nova explodes, you all will burn in Hell.

Some of us refuse to believe in Mother Nature. We say to Mother Nature that you are too old, too weak, and too slow. You have taken four and a half billion years for us to arrive on Earth, and we arrived on Earth only three and a half million years ago. You wasted so much of time that the Sun has used up more than half of its energy. We send Mother Nature to retirement home and seize power from her, and we speed up evolution. If the Moons in our Solar system like Enceladus and Europa are confirmed Water World, we could send genetically modified earliest life forms like Blue Green Algae to spread like carpet their surface. Billions of Algae's spores will carry extra genes which make them resistant to heat, cold, desiccation. They will carry additional Chloroplast Genome. The transgenic Algae replicate and double every twenty minutes. Within a few centuries, the Blue Green Algae will carpet the surface of the Moons. Its job is to suck Carbon dioxide and release Oxygen. We will monitor its progress by analyzing the sunrays deflected from the surface of their Moons during eclipse identifying spectral peaks for Oxygen, Nitrogen and Carbon dioxide. If we succeed in harvesting genetically modified life on the surface of the Moons of our Solar System, we could send hundreds of unmanned spacecrafts to the Water Worlds of distant Star Systems. Some of us leave this Solar System before it becomes Super Nova. We learn for the first time that Humanity has a collective responsibility. We will prove that our fate is not extinction, but expansion, protection, and preservation of human intelligence to spread in every corner of the Universe.

What is the fate of humanity on Earth? We have no future on Earth. Humanity is trapped in a middle age dying Solar System. Our Sun has been burning for the past four and a half billion years. The fusion of Hydrogen to Helium to produce energy; it uses 70 million tons Hydrogen every second. The release of the subatomic particles such as photons by the fusion of Hydrogen atoms come to us as sunlight. Over four and a half billion years, our sun has used up more than half of its total energy. It is a pale-yellow middle age dying star. During the next five billion years, the Sun will continue to burn and exhaust its energy and convert all its Hydrogen to Helium to its core and will swell to become the Red Giant. Now, we realize that we are trapped in a middle age dying Solar System. As it cools, the Sun will expand and will allow the nearest two rocky planets Mercury and Venus and they will melt and then become a part of Red Giant. As it expands further, its outer rim approaches Earth. The intense heat of millions of degrees centigrade will boil off our oceans and melts and evaporate mountains. The intense heat will incinerate plants, animals and kill all life forms on Earth. As more and more energy are used up, the Red Giant has exhausted most of its energy and it will expand no farther; it will collapse on itself and then will explode as Super Nova. How could we safe humanity from total annihilation. If human intelligence extinguishes from Earth, no one in the entire Universe will ever know that intelligent life ever existed in this corner of the Universe. Scientists wonder if intelligent life exists anywhere in the Universe and whether they would know if any kind of Life ever existed on Earth.

Planet Earth is one of the eight satellites of our star Sun. Our Earth received over hundred Terawatts of energy each day from our Sun. About twenty percent of the energy, we use to run the engine of modern society. Earth recycles food, water, and air to maintain life. If we want to prevent life from extinction before Super Nova explosion, humanity must leave our Solar System; we need to develop fleets of city size spacecrafts and place them in suitable orbits of some distant energy providing star systems. Our science and technologies are being developed so rapidly, if we don't destroy ourselves by going to nuclear war for the next millennium, (the following nations including the USA, Russia, the UK, France, China, India, Pakistan, and Israel together possess a total of approximately 19 000 nuclear weapons), we have enough time to develop technologies to leave this Solar System. Once we learn to recycle our food, air, and water we could travel to unknown destination to find a suitable star system and place our satellites in an appropriate orbit that is in the habitable zone or Goldie Lock Zone. During the past fifty years of our Space Program, we have made enormous progress. Before this century is over, we must develop enough technologies to colonize planet Mars. It is expected that Orion Spacecraft will take the crew of NASA to Mars by years 2030. The experience gained would teach us the solution to our many problems on the long journey to unknown destination for many people.

Besides the major question, are we alone? Three important questions raised by the scientists of the last century are answered now: (1) what is Life, (2) what is conscientiousness, and (3) what is the destiny of humanity on Earth? Our answer to the first question is that Life is a DNA carrying biological machine which is based on the interactions of four information molecules called nucleotides. They are A-T (Adenine-Thiamine) and G-C (Guanine-Cytosine). With enzyme DNA polymerase, restrictions enzymes, and enzyme DNA ligase, we cut, paste, sequence, and copy these molecules. Obeying the laws of Physics, Chemistry and Biological Evolution, they organized themselves to become alive. Using these four molecules, we created artificial life form. Using computers, we designed the simplest life form and using A-T and G-C we created the design of an artificial life form. Our aim is not to create humans, but to create mindless, brainless biological machines to solve our immediate problems facing humanity. These biological machines will carry instructions: (i) to clean up our environmental pollution, (ii) to provide new food for the burgeoning population of the world, (iii) to provide new fuel to run the engine of the modern society, and (iv) to provide new medicine to treat every disease known to mankind.

Our answer to the second question is that conscientiousness arose in Africa. Although Chimps were living in Africa for the past 26 million years, (confirmed by the fossil records) about three million years ago in the brain of a Chimp known as Lucy, a few genes are turned on giving her conscientiousness. Radioactive dating showed that about three million years ago Lucy was the first human/chimp who walked on her two feet, and she was identical to modern human in her skeletal structure. She was the first Chimp/human who became aware of her surrounding and environment and gained conscientiousness. Genetically, Chimps are 98.9% identical to Lucy. She was the first Homo erectus who could walk on her two feet. Her children walked out of Africa in search of food, water, and shelter and within 100,000 years they spread all over seven continents and in 200 nations. Today more than seven and a half billion of them live on Earth and they are all brothers and sisters' children of the same mother a black woman, Lucy, was born in Africa about three million years ago.

What is the Destiny of Mankind?

What is the destiny of mankind? One thing is certain that we cannot live on this planet forever. The sad part is that we arrived at the scene so very late. As I said above, the Sun has been burning for the past four and a half billion years. It burns seven million tons of Hydrogen gas every second to produce Helium. It has used up half of its energy. As the Sun cools, it expands to sallow Planets Mercury and Venus and as it approached Earth, the shear heat would evaporate Oceans, burn, and char trees and turn to ashes every living creature on Earth. Eventually, the molten Earth will become a part of the burning Sun. The new Sun will become so large that it will collapse on itself and exploding as the Super Nova destroying the entire Solar System.

Humanity has come on a crossroad. We have an option, either to sit on our hands and do nothing and accept our faith and we all die when the Sun dies or to escape Earth before it becomes Super Nova and explode. The collective responsibility of every man, woman and child on Earth is to preserve, protect, and spread human intelligence in every corner of the Universe. If you relax by saying that we still have plenty of energy in our Sun to keep us alive for quite some time, we are gravely mistaken. What if we destroy ourselves by going to nuclear war or cause environmental collapse by polluting Earth or infected by a new viral infection like the Spanish Flu or COVID-19 (within two years, Coronavirus has killed over five million people worldwide) and the Spanish flu wiped out one third of world population within months during last century. There is no guarantee of our survival forever. Even if we don't destroy ourselves by infighting, what about the external dangers to our life on Earth, we live in a shooting gallery of comets and asteroids. We have no time to sit on our hands and do nothing. If human intelligent life extinguished from Earth, it would never evolve again on Earth. There is not enough energy left in our solar system for the life to evolve once again. If human intelligent extinguish on Earth, no one in the entire Universe will ever know that intelligent life ever existed in a tiny Solar System on the third arm of the Milky Way Galaxy.

How Safe Are We?

You could say that we are very safe if our Sun continue to burn for at least another four and a half billion years. You could say that we can live in peace and tranquility for a long time. You are right, but have you heard of Asteroid/Kuiper Belt in the inner Solar System where billions of huge rocks orbiting our Solar System. Once a while a comet like Haley's which orbit our Sun every 75 years, or orbiting planet like Jupiter or passing Star perturbs some of those Asteroids and they hurtle in every direction including inwards in the direction of Earth. If any of the planets of our Solar System is in on its path, they will hit with a devastating force.

For example, in June of 1908, in a quiet forest in Siberia, Russia, an Asteroid exploded in the atmosphere with force hundreds of times more powerful than the Hiroshima Bomb destroying the forest in a thousand-mile radius now known as the Tunguska event. The explosion released 12 megatons of energy. Was it a rare event or could it happen again? If we only look at the surface of the Moon, we find it is marked by thousands of craters and by thousands of meteorites. These craters are preserved due to the lack of atmosphere on the Moon. A mile wide creator in Arizona thought to be a volcano was reexamined and was determined that it was an impact crater caused by an Iron meteorite. Using the new technologies, Geologists have determined that there are more than 200 craters on Earth, and they are all made by Asteroid impact. Another example, in 1975, the greatest mystery of the extinction of Dinosaurs who died 65 million years ago was solved by a father/son team of Alvarez who found that the layer of Carbon around the globe contains a high concentration of Iridium an element rare on Earth but common on Asteroids. They believed that a huge Asteroid struck near the coastal Mexican town of Chicxulub Puerto 66 million years ago. (The asteroid that struck new Chicxulub Puerto was ten miles wide and forever changed history when it crashed into Earth about 66 million years ago. The Chicxulub impactor, as it's known, was a plummeting asteroid or comet that left behind a crater off the coast of Mexico that spans 93 miles and goes 12 miles deep.) Their theory was confirmed during an aerial survey for the oil deposit when huge symmetrical rings of highly magnetic material was discovered which was more than one hundred miles across buried beneath a mile under the surface. It was unmistakable mark of a deep impact crater.

You might still say that it was so long ago. Let us take the most recent example. On March 23, 1993, a multimillion mega ton Asteroid named Levy/Shoemaker crashed on the surface of the planet Jupiter. As Asteroid entered the Jupiter's atmosphere, it was broken by the Jupiter's gravitational force into 21 pieces and all the pieces were on their collision course on the surface of Jupiter. Fourteen hundred miles high above the surface, the Hubble Telescope photographed the event. One by one each piece crashed and exploded on the rotating Jupiter's surface. Jupiter is the largest planet in our Solar System. It can accommodate a thousand Earth inside its volume and could accommodate the impact shock easily.

After traveling for over 15 months, the fragments reached the surface of Jupiter. On the night of July 16, 1994, a series of cosmic collision occurred, and the first fragment called "Nucleus A" crashed on Jupiter. One by one the Asteroid's fragments crashed on the Jupiter's surface. The damage inflicted on the Jupiter's surface was incredible. Few days later, the biggest fragment, "Nucleus G" smashed on the surface with a force half a million times greater than the Tunguska explosion which is 12 megaton explosions. It produced a Big Black spot. "Nucleus G" released six million megatons of energy on Jupiter.

If such a collision had occurred on Earth, it would have been a catastrophe for our planet. Its impact would be devastating. The oceans would have evaporated; forests including all life forms would have burned to ashes. It would cause massive Earthquakes; generate huge firestorms; everything would be burned and charred. The Big Black spot-on Jupiter would have become the Big Black Planet Earth. The Solar System would still be there, but its third

planet would be Big Black lifeless planet. If life is extinguished on Earth, it would never evolve again. How safe are we? The answer is not very. No matter how tranquil the life is on Earth, it is temporary.

To save mankind from destruction, we must leave this Solar System. Our destiny lies in another Star System not here. Travelling with a speed of 186,000 miles per second, Light takes about 28 trillion miles to travel for one year called a Light year. Twenty years ago, Europeans Radio astronomers have identified one Earth like planet called 51 Pegasi within ten light years. The New Space Telescope, Kepler, detected more than 4,000 Earth like planets, called exoplanets and found that forty percent of all stars have multiple planets like ours. Kepler also discovered that Planets revolving around a star is common. Most Planets are either half the size of Earth or twice as large. We need to train an army of young scientists to travel on a one-way journey first to the nearest star called Alpha-Century which is about four and a half light year from Earth. It is a binary Solar System. It is expected that there would be more than one Earth like planets.

To take the humanity out of the dying solar system, our priority is to learn to recycle our food, our water, and our air for the fleets of city-sized satellites carrying humans to unknown Star Systems. For decades, the International Space Station has been trying to become self-sufficient. One day, it will be successful and become independent. As an independent Space Colony, it could travel long distances in search of habitable planets.

About a quarter of a century ago, Dr. Daniel Golden, the former director of NASA, provided a vision for the fate of humans on Earth to explore distant Star Systems for habitable planets and to take the humans out of this dying Solar System. Dr. Goldin served as the 9th and longest-tenured Administrator of NASA from April 1, 1992, to November 17, 2001. He was appointed by President George H. Bush and served under President Bill Clinton and George W. Bush.

By April 14, 2003, when we (scientists at NIH) were about to complete the Human Genome Project, he called for a meeting between scientists from NIH and NASA. National Institutes of Health (NIH) is the largest Biomedical Research Center in the World. We are located about 10 miles from Washington, DC. Our annual budget is over $41 Billion. Twenty-one thousand best and brightest scientists are assembled from around the world. They work in 27 different institutes in over three thousand labs. For the past one hundred years, NIH has become a citadel of research and learning. It is home to some of the greatest minds in the world. More Nobel Laureates walk through the streets of NIH than anywhere on the face of the world. The discoveries they make here benefit more than seven and a half billion people around the world.

Dr. Goldin challenged scientists in both agencies. He said that he had a dream to land men on Planet Mars before this century is over. Not just land men on Mars, but to colonize Mars and used Mars as a base to launch unmanned spacecraft in search of Earth like planets, a second Earth, to transport humans into a new and safer environment. To succeed in this endeavor, he proposed the following three assignments for scientists at NASA and three assignments for scientists at NIH. For NASA, he proposed the following: 1) Using hardest and lightest material (like Graphene recently obtained from Graphite) to build a fleet of city size spacecrafts to transport humans to other star systems; 2) Using fusion reaction which generates energy on Sun, construct a machine to produce unlimited amount of clean energy (recently done at Princeton University Princeton Plasma Physics Laboratory (PPPL) which is a United States Department of Energy national laboratory for plasma physics and nuclear fusion science. It will generate massive amounts of energy that could provide a virtually inexhaustible supply of power to generate electricity and 3) Using supersonic technology accelerate the speed of spacecrafts to gain at least half the speed of light.

While NASA's main function will be working on manufacturing super spacecrafts and super telescopes, scientists at NIH are asked to help astronauts in the following three areas: Dr. Golden said he cannot send a fleet city size spacecrafts full of travelers on a one-way journey in search for a habitable planet on new Star System who is suffering from pain and diseases. He asked to us find genes which are responsible for causing pain and suffering. He also cannot send astronauts on a long journey who are suffering from diseases. He asked us to find genes which are responsible for causing diseases and finally, he cannot send astronauts on a possible one-way journey if they live only a hundred year. We are to find genes which are responsible for aging. He has inspired scientists at NIH as we have never been inspired before.

We describe below how much both agencies have accomplished so far:

NASA'S Accomplishments

NASA accomplished miracles by sending a series of Mars rovers. Over the years, NASA has sent five robotic vehicles, called rovers, to Mars. The names of the five rovers are: Sojourner, Spirit and Opportunity, Curiosity, and Perseverance. In January 2004, two robotic geologists named Spirit and Opportunity landed on opposite sides of the Red planet. With far greater mobility than the 1997 Mars Pathfinder rover, these robotic explorers have trekked for miles across the Martian surface, conducting field geology tests and making atmospheric observations.

After the success of Hubble Space Telescope came NASA's The Kepler Mission which is specifically designed to survey our region of the Milky Way galaxy to discover hundreds of Earth-size and smaller planets in or near the habitable zone and determine the fraction of the hundreds of billions of stars in our Milky Way galaxy that might have such planets. Kepler has discovered the first Earth-size planet orbiting a star in the "habitable zone" – the range of distance from a star where liquid water might pool on the surface of an orbiting planet. The discovery of Kepler-186f confirms that planets the size of Earth exist in the habitable zone of stars other than our sun. Kepler-186f resides in the Kepler-186 system, about 500 light-years from Earth in the constellation Cygnus. The system is also home to four companion planets, which orbit a star half the size and mass of our sun. Although Kepler mission is over and it will shut down its radio transmitter and onboard fault-protection systems, becoming an inert chunk of metal that floats, silent and un-responding, through the cold, dark depths of space. Kepler is currently trailing the Earth by about 94 million miles and will remain the same distance from the Earth for the foreseeable future.

To replace Kepler, on December 18, 2021, NASA will launch its most powerful telescope called. The James Webb Space Telescope. It will be the world's premier space science observatory. The Webb Telescope will solve mysteries in our solar system, look beyond to distant worlds around other stars, and probe the mysterious structures and origins of our universe and our place in it.

NIH'S Accomplishments

Within less than a quarter of century, I am pleased to report that we at NIH have made enormous progress in all three areas. We sequenced the Human Genome; we have identified 24,000 genes. We identified 16,000 good gene and six thousand bad genes responsible for six thousand different diseases. We have also identified two thousand pseudogene who lost their function. We have also identified about half a dozen mutated endorphin genes which are responsible for causing pain and suffering. We provided funds to researchers who discovered the Aging genes which could double human age and for their discovery 2009 Noble Prize in Medicine shared jointly by Elizabeth H. Blackburn, Carol W. Greider and Jack W. Szostak "for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase." Eternal youth was the dream of every King, Queen and Clown. Now, it is becoming a reality. Cancer cells do not grow old and die while the normal cells do. Scientist discovered that the tip end of each chromosome carries hundreds of six letter codes (TTAGGG) called the telomeres. As cells divide and grow, they lose dozens of telomeres. Aging in normal cells is an internal combustion due to loss of telomeres. On the other hand, cancer cells do not loose telomeres at the same rate. The genes in cancer cell which prevents aging called telomerase reverse transcriptase has been identified and isolated. Viruses could serve as vector for carrying the gene Human telomerase reverse transcriptase for doubling the human age. Although the work on humans is not permitted at this time, it could be a possibility within the next hundred years.

Years ago, Dr. Goldin's speech was considered outlandish; today it is a doable reality.

NIH Contribution to Future Space Travelers

What NIH could contribute to NASA's effort in search for life beyond our Solar System? NIH is the largest biomedical center in the world. Its current function is to take care of health and wellbeing of all humans. NIH has been my home for more than a quarter of a century. Our role is to conduct research, support research and report research. The discoveries made at NIH benefits all mankind. Our future role would include working on all living systems that are sent on a long journey in search of habitable planets in distant star system including creatures such as plants, animals, and humans.

To understand the basis of all diseases, we must read and understand the total genetic information that makes us that is to read our genome which is our normal book of life. That is how the story of our book of life begins: As we all know that we are the loving union of our parents. Our mother's egg receives our father's sperm, and we are conceived. The fertilized egg carries complete information to make us. More than seventy years ago, the Nobel Laureate, Irvin Schrödinger, examined for comparison, the fertilized egg of a human, mouse, and monkey under a microscope. He observed that all fertilized eggs look the same and yet first fertilized egg carries the instructions to make a man, the second carries the information to make a mouse and third carries the information to make a monkey. He postulated that there exists a secret code within those fertilized eggs; he called that secret code, the Script Code now known as the Genetic Code. He stated that if we break the Genetic Code, we would be able to unlock the secret of life. If we unlock the secret of life, we would be able to understand how the normal cells become abnormal that is cancerous.

On further examination of the fertilized egg, we found that the essence of life is information and information is located on four molecules called nucleotides bases. DNA (Deoxy ribonucleic acid) is made of a string of nucleotides and is a store house of information and is made of the same four nucleotide bases and they are: Adenine (A), Thymine (T), Guanine (G), and Cytosine (C). According to Crick's Central Dogma the information flows from the double stranded DNA which is transcribed into a single stranded RNA which is translated in Ribosome into proteins [1]. RNA is converted into an active form and is transcribed into m-RNA (or messenger RNA, by deleting intervening non-coding nucleotides called splicing) by converting Thymine to active form Uracil (U) and from a double stranded DNA to a single stranded RNA and where the sugar Deoxy Ribose is replaced by sugar Ribose. The RNA is translated by Ribosome into proteins.

Gene Expression begins in Ribosome when a 4-letter genetic text is converted to a three-letter code called Codon. By comparing Gene Profiles of normal genes with mutated genes, one can identify with precision and accuracy the exact location of mutated (altered or damage) nucleotide responsible for causing the disease. Comparing Gene Profiles is an excellent diagnostic method which helps us design drugs to specifically shut off the mutated genes. Seventy years ago in the above experiment, Schrödinger was using such a poor resolution microscope that we don't even use in our high school today. Instead, we have electron microscope. We can magnify the same fertilized egg to a million times of its original size, almost the size of a house. What we observe inside the fertilized egg is very analogous to the house. The house has a kitchen; the cell has a nucleus. Suppose your kitchen has a shelf which contains 46 volumes of cookbooks which contain 24,000 recipes which carry instructions to cook food for your breakfast, lunch, and dinner. The nucleus in the fertilized egg contains 46 chromosomes; (23 chromosomes from our mother and 23 from our father), which carry 24,000 chapters called genes. Genes are units of inheritance which code for all 20 amino acids. Hundreds of amino acids join to form a protein and thousands of proteins interact to make a cell. Millions of cells interact to make an organ and several organs interact to make a man or a mouse or a monkey. The number and the order of the nucleotides determine the composition of a species.

If the cookbook in your kitchen is written in English language, it uses 26 letters, but the book of life of all living creatures is written in 4 letters and they are A, T, G and C. These are the initials of four chemicals called nucleotides (Adenine, Thymine, Guanine and Cytosine) found in the nucleus of all living cells. Nucleotides are made of sugar Ribose (Deoxy Ribose in DNA and Ribose in RNA), a phosphate group and one of the four Nitrogen bases, two Purines and two Pyrimidines and the Thymine is converted to Uracil in RNA. These molecules are found in the nucleus of all living cells from a tiny blade of Grass to mighty elephant including man, mouse, and monkey. The total genetic information to make any living creature is based on the above four-letter text and out of these four letters, only three letter Codon which carries the Genetic Code for an amino acid (such as GUU is for amino acid Valine, GCU is for Alanine, GAA is for Glutamine etc.) the building blocks for all proteins. Sixty-four codons code for 20 amino acids and codons for all 20 amino acids have been decoded. All living creatures use the same genetic code. A string of these nucleotides is called the DNA (Deoxy Ribonucleic Acid). Reading the number and the order of nucleotides are called genome sequencing. The total genetic information to make a species is called its Genome.

In 1990, United State Congress authorized three billion dollars to NIH to decipher the entire Human Genome within 15 years that is the total genetic information that makes us human called the Human Genome Project. Thousands of scientists from six industrialized nations and 20 biomedical centers joined our effort and within 13 years the entire human genome was deciphered and published in the Scientific Journal Nature and linked to website. If you have access to a computer keyboard, you have access to all that information.

The Human Genome Project

On April 14, 2003, Dr. Francis Collins, the Director of NIH announced that we read the book in which God created life. We read the book of life of a human being letter by letter, word by word, sentence by sentence and chapter by chapter. All 46 volumes (Chromosomes) containing 24,000 chapters (Genes) consisting of six billion four hundred million letters. In a few sentences, the described the Human Genome Project, the greatest biological experiment ever conceived by human mind. It will answer the most fundamental questions we have asked ourselves since the dawn of civilization, what does it means to be human, what is a nature of our memory and conscientiousness and our development from a single cell to a complete human being, the biochemical nature of our senses, the process of aging. The scientific basis of our similarity and dis-similarity; similarities that all living creatures from a tiny blade of grass to a mighty elephant including man, mouse and monkey are all made of the same chemicals building blocks and yet they are so diverse that no two individuals are alike even identical twin and not identical, they grow up to become to separate individual.

We deciphered all 46 chromosomes. What surprised us most was that our genome contains six billion four hundred million nucleotide base pairs less than two percent of our Genome contains genes which code for proteins. The other 98 percent of our genome contains switches, promoters, terminators etc. Before sequencing (determining the number and the order of the four nucleotide), it is essential to know how many genes are present in our Genome. The Human Genome Project has identified the following genes on each chromosome.

We found that the chromosome (1) is the largest chromosome carrying 263 million A, T, G and C nucleotides bases and it has only 2,610 genes. The chromosome (2) contains 255 million nucleotides bases and has only 1,748 genes. The chromosome (3) contains 214 million nucleotide bases and carries 1,381 genes. The chromosome (4) contains 203 million nucleotide bases and carries 1,024 genes. The chromosome (5) contains 194 million nucleotide bases and carries 1,190 genes. The chromosome (6) contains 183 million nucleotide bases and carries 1,394 genes. The chromosome (7) contains 171 million nucleotide bases and carries 1,378 genes. The chromosome (8) contains 155 million nucleotide bases and carries 927 genes. The chromosome (9) contains 145 million nucleotide bases and carries 1,076 genes. The chromosome (10) contains 144 million nucleotide bases and carries 983 genes. The chromosome (11) contains 144 million nucleotide bases and carries 1,692 genes. The chromosome (12) contains 143 million nucleotide bases and carries 1,268 genes. The chromosome (13) contains 114 million nucleotide bases and carries 496 genes. The chromosome (14) contains 109 million nucleotide bases and carries 1,173 genes. The chromosome (15) contains 106 million nucleotide bases and carries 906 genes. The chromosome (16) contains 98 million nucleotide bases and carries 1,032 genes. The chromosome (17) contains 92 million nucleotide bases and carries 1,394 genes. The chromosome (18) contains 85 million nucleotide bases and carries 400 genes. The chromosome (19) contains 67 million nucleotide bases and carries 1,592 genes. The chromosome (20) contains 72 million nucleotide bases and carries 710 genes. The chromosome (21) contains 50 million nucleotide bases and carries 337 genes. The Chromosome (22) contains 56 million nucleotide bases and carries 701 genes. Finally, the sex chromosome of all females called the (X) contains 164 million nucleotide bases and carries 1,141 genes. The male sperm chromosome (Y) contains 59 million nucleotide bases and carries 255 genes [2-6].

If you add up all genes in the 23 chromosomes, they come up to 26,808 genes and yet we keep on mentioning 24,000 genes. The remaining genes are called the pseudo genes. They are broken genes that have lost their functions. For example, millions of years ago, humans and dog shared some of the same ancestral genes; we both carry the same olfactory genes. Since humans don't use these genes to smell for searching food, these genes are broken and lost their functions in humans, but we still carry them. We call them Pseudo genes. Recently, some Japanese scientists have activated the pseudo genes, this work may create ethical problem in future as more and more pseudo genes are activated. The above DNA nucleotide bases constitute the genetic map of the normal human being what makes it abnormal and makes us sick is the mutations in the coding regions of the genome. As I said above, less than two percent of the genome codes for amino acids. Slightest damage to the coding regions of the four nucleotides A, T, G and C either by radiations, or chemical pollution or genetic inheritance or viral infection or Errors occur during rapid replication such as insertion, deletion, or inversion of the nucleotide bases coding for wrong or abnormal amino acids resulting in diseases.

A gene is a strip of DNA which has a start codon AUG (codes for amino acid Methionine) and has three stop codons and they are UGG, UGA, UAG. Out of 24,000 genes, 16,000 are good genes which produce good proteins that keep us healthy. These genes produce protein like Insulin for treating diabetics; thousands of scientists are working in about three thousand biotechnology firms producing good proteins. There are about six thousand bad genes which are mutated, and they are responsible for causing six thousand different diseases. Single genetic defect can be treated with the Gene Therapy by replacing the bad genes with the good genes as it is successfully accomplished in SCID (Sever Combined Immuno-Deficiency) Syndrome. Most diseases are due to multiple genetic defects. They cannot be treated with Gene Therapy, but Drug Therapy will work. Treating multiple genetically defected diseases with novel drugs is a laborious and expensive process. It requires a series of safety and efficacy test before it goes for clinical trials in humans.

By the turn of this century, NIH will ensure that the space travelers will have their genome done and they will carry with them their genomic profile on a computer chip. We should have developed the sequenced data base of all individuals. All mutated genes should have been identified and removed from the gene pool of fertilized eggs before conception. Gene Therapy and Drug Therapy would have taken care of escaped mutated genes.

The cost of sequencing will come down slowly as we develop next generation sequencers like Parallel or Nanopore sequencers. Within a few years, we all should have sequenced our genome and download on a computer chip which we will carry with us all the time. In case of medical emergency, the clinical staff will sequence your current genome and compare with your genome chip and identify the problem and provide medical help instantly.

Cheaper sequencing could create ethical problem such as human cloning or organs harvesting. For example, an unmarried woman could create her own clone by taking a single nucleus from her primordial stem cell whose all 24,000 genes are functioning and transfer its genome in her own egg whose nucleus has been removed. She could incubate the clone in her won womb and give birth to her own clone in nine months. Similarly, we could grow various organs for transplant by sequencing the genomes of Liver, Lungs or Kidneys and harvest in tissue culture for future transplant. (We need to develop ethical guidelines for the physicians).

Our next accomplishment includes the completion of The Multiple Genomes Projects: The 1000 Genomes Project is an international research consortium that was completed in 2007 whose aim was to sequence the genomes of at least 1,000 volunteers from multiple populations worldwide to improve our understanding of the genetic contribution to human health and disease. During 2018, The British Government completed the 100,000 Genomes Project of the British citizens managed by Genomics England that is sequencing whole genomes from National Health Service patients. The project is focusing on identifying rare diseases, some common types of cancers, and infectious diseases in British population. The Chinese are working on a million-genome project.

Space Medicine

For the citizens of Cosmos, who are willing to go on a one-way trip in search of a habitable exoplanet, we must find treatment for all diseases caused by all the six thousand mutated genes. Before they get onboard for the endless journey, we must check for any signs and symptoms of all common as well as rare allele diseases.

Drug Design for Common Allele Diseases

These are the diseases of people of all ages. Before the development of antibiotics, most people died of infectious diseases around age 50. First, antibiotics, Penicillin, (discovered by Alexander Fleming) was used for treating wounds before the WWII. As I said above, enormous funds were made available by the Army to develop large scale antibiotics to treat wounded soldiers returning from the battle ground during WWII. During the following decades, novel class of aminoglycoside antibiotics were discovered which are valuable therapeutic agents. Some of them are Streptomycin, Neomycin, Kanamycin, Paromomycin, Apramycin, Tobramycin, Amikacin, Netilmicin, Gentamicin. etc. Dozens of their water/fat soluble derivatives were synthesized. They are considered broad spectrum antibiotics because they inhibit the growth of both Gram-negative and Gram-positive bacteria causing deadly diseases and save human life. All aminoglycoside antibiotics are relatively small, basic, and water-soluble molecules that form stable salts. Most aminoglycoside antibiotics are products of fermentation of filamentous actinomycetes of the genus Streptomyces.

Drug Design for Rare Allele Diseases

Diseases of old age people includes Cancers, Cardiovascular diseases, and Alzheimer. Now a day, people rarely died of infectious diseases. Because of the availability of a variety of antibiotics, today, most people live beyond age 70 years and some of them go on living beyond 80 years of age. Those who live beyond 70 are faced with three major old age diseases which are responsible for causing the death of most patients during their lifetime and they are Cancers, Cardiac Diseases and Alzheimer. These are genetic diseases and could be treated either by Gene Therapy (using CRISPR technology by replacing bad gene with the good gene using CRISPER-Cas9) or by Drug Therapy. There are about three thousand monogenic diseases and could be treated by replacing the defected gene with good gene that is by Gene Therapy or designing drugs to shut off the bad genes that is Drug Therapy. Gene Therapy cannot be applied to treat multiple genetic defects such as Alzheimer, Cancers, or cardiovascular diseases. Drug Therapy could be used to develop novel treatments. Recently completed 1,000 Human Genome Project identify with precision and accuracy the genes responsible for causing these diseases. It is now possible to design drugs to shut off these genes and save human life. Genes code for Proteins and a mutated gene codes for abnormal proteins resulting in these diseases.

Cancers

Cancer is the leading cause of death and has surpassed the death of cardiovascular diseases. Over 636,000 people died of cancer; 1.9 million new cases will be diagnosed this year including 78,000 Prostate Cancer, 40,000 Breast cancer, 16000 Lung and Bronchus Cancer and 15,000 Colon and Rectal Cancer. Once diagnosed by Gene Sequencing, the next step is to design drug to shut off those genes.

The Rational Drug Design to Treat Cancers

All three old age diseases that is Cancer, Cardiovascular Diseases and Alzheimer carry multiple mutated genes responsible for causing these diseases. In each of the above three diseases, it is the mutated genes that code for wrong protein which causes these diseases. If we design drugs to shut off mutated genes in one disease, using the same rationale, we should be able to shut off bad genes in all three old age diseases. Although Coronary Artery disease is a complex disease, researchers have found about 60 genomic variants that are present more frequently in people with coronary artery disease. Most of these variants are dispersed across the genome and do not cluster on one specific Chromosome. To shut off a bad gene, DNA binding agents are developed. DNA binding Nitrogen Mustard is highly toxic. The other non-toxic DNA binding agents like Aziridines and Carbamate serve as a prodrug. They must be activated in the acidic medium. Drugs are designed to seek out the specific malignant gene which replicate faster producing acids. Aziridines and Carbamate moieties are sensitive to acid. Drugs carrying the Aziridines, and Carbamate's moieties are broken down in acidic media generating Carbonium ions which attack DNA shutting off genes. Only the acid producing genes will be attacked no matter where they are located. It does not matter whether they are clustered or dispersed across genome.

The supreme intellect for Drug Design is Ross, an Englishman, who is a Professor of Chemistry at the London University, England. Professor WCJ Ross is also the Head of Chemistry Department at the Royal Cancer Hospital, a post-graduate medical center of the London University. Ross was the first person who designed drugs for treating Cancers. He designed drugs to cross-link both strands of DNA that we inherit one strand from each parent. Cross-linking agents such as Nitrogen mustard are extremely toxic and were used as chemical weapon during the First World War (WWI). More toxic derivatives were developed during the Second World War (WWII). Using the Data for the toxic effect of Nitrogen Mustard used during the WWI, Ross observed that Soldiers exposed to Nitrogen Mustard showed a sharp decline of White Blood Cells (WBC) that is from 5,000 cell/CC to 500 cells/CC. Children suffering from Childhood Leukemia have a very high WBC count over 90,000 cells/CC. In sick children, most of the WBCs are premature, defected, and unable to defend the body from microbial infections. He also observed a sharp decline in the energy producing Mitochondria. Ross rationale was that cancer cells divide faster than the normal cell, by using Nitrogen Mustard

to cross linking both strands of DNA, one can control and stop the abnormal WBC cell division in Leukemia patients. It was indeed found to be true. Professor Ross was the first person to synthesize hundreds of derivatives of Nitrogen Mustard. By using an analog of Nitrogen Mustard, called Chlorambucil, he was successful in treating Childhood Leukemia. In America, two Physicians named Goodman and Gilman from the Yale University were the first to use Nitrogen Mustard to treat cancer in humans. Nitrogen Mustards and its analogs are highly toxic. Ross was a Chemist, over the years, he synthesized several hundred derivatives to modify toxicity of Nitrogen Mustard [7].

Although analogs of Nitrogen Mustard are highly toxic, they are more toxic to cancer cells and more cancer cells are destroyed than the normal cells. Toxicity is measured of the Chemotherapeutic Index (CI) which is a ratio between toxicity to Cancer cells versus the toxicity to Normal cells. Higher CI means that the drugs are more toxic to cancer cell. Most cross-linking Nitrogen Mustard have a CI of 10 that is they are ten times more toxic to cancer cells. Some of the Nitrogen Mustard analogs Ross made over the years are useful for treating cancers such as Chlorambucil is used for treating childhood leukemia (which brought down the WBC level down to 5,000/CC). Children with Childhood Leukemia treated with Professor Ross Chlorambucil showed no sign of Leukemia even after 20 to 25 years later. Chlorambucil made Ross one of the leaders of the scientific world. In addition, he also made Melphalan and Myrophine for treating Pharyngeal Carcinomas [8-10].

At the London University, I was trained as an Organic Chemist in the Laboratory of Professor WCJ Ross of the Royal Cancer Hospital, a post- graduate medical center of the London University. I graduated from London University. After finishing my doctoral and postdoctoral studies, I became a permanent staff in Professor Ross' Lab. As I said above, Professor Ross was designing drugs to attack both strands of DNA simultaneously by cross-linking using Nitrogen Mustard analogs, which are extremely toxic. As a part of my doctoral thesis, I was assigned a different path. Instead of cross-linking DNA, I am to design drugs to attack only one strand of DNA. This class of drugs is called Aziridines. Over a ten-year period, I made over 100 Dinitrophenyl Aziridines derivatives. One of them is Aziridine Dinitro benzamide (CB1954) which gives a CI of 70 highest ever recorded. CB1954 wipes out a solid tumor by attacking the DNA of Walker Carcinoma 256, a solid aggressive tumor in Rat. Nitrogen Mustards are highly toxic because they have neither specificity nor selectivity. They attack all dividing cells whether they are normal or abnormal. On the other hand, the analogs of Aziridines and Carbamates serve as Prodrugs remain inactive in the basic and neutral media. They become activated only in the presence of Acidic media produced by cancer cells.

I used a simple rationale, the Aziridine attacks DNA in acidic medium, particularly the N-7 Guanine. The dye Dinitro benzamide has great affinity for Walker Tumor [11-13]. The Aziridine dinitro benzamide (CB1954) stain the tumor. As the tumor grows, it uses Glucose as a source of energy. Glucose is broken down to Pyruvic Acid. It is the acid which attacks the Aziridine ring. The ring opens to generate a Carbonium ion which attacks the most negatively charged N-7 Guanine of DNA shutting off the Walker Carcinoma gene in Rat. To continue my work, I was honored with the Institute of Cancer Research Post-Doctoral Fellowship Award of the Royal Cancer Hospital of London University. To increase the toxicity of CB1954 to Walker Carcinoma, I made additional 20 analogs as a postdoctoral fellow. When I attached one more Carbonium generating moiety, the Carbamate moiety to the Aziridine Dinitrobenzene, the compound Aziridine Dinitro benzamide Carbamate was so toxic that its Therapeutic Index could not be measured. We stop the work at the London University for the safety concern [14-16].

After working for about ten years at the London University, I moved to America when I was honored by the Fogarty International Postdoctoral Fellowship Award by the National Institutes of Health, NIH, and the National Cancer Institute, NCI, of the USA. NIH has been my home for over a quarter of a century, I designed drugs to shut off mutated genes. All three Common Allele diseases (Cancers, Cardiovascular and Alzheimer) have genetic origin. The rationale I used to synthesize anti-cancer drugs could be used rationally designed novel drugs to treat the other two old age diseases like Alzheimer or cardiovascular diseases. In the following sections, I will describe in detail how anti-cancer drug like AZQ was designed to shut off Glioblastoma genes which cause Brain Cancer in humans. Using the same rational, we will consider how each of the other two diseases namely cardiovascular disease and Alzheimer could be treated by shutting off their genes to save human life: The order of these diseases is arranged based on the level of funding provided by NIH specifically by the NCI (National Cancer Institute).

I continued my work by synthesizing the highly toxic Aziridine/ Carbamate combination analogs in America. I brought the idea from London University of attacking one strand of DNA using not only Aziridine, but also Carbamate without using the same dye Dinitro benzamide. I was brought to NCI to translate animal work to human.

Historical Background of Glioblastoma

Our Brain is a three-pound flesh that you can hold in the palm of your hand. It can contemplate the vast distances among billions of Galaxies across Universe; It can contemplate the concept of Infinity. It convinces us to believe in existence or non-existence of God. It questions our Ethics; our Morality, our Altruism and our Free-will. It is only a three-pound flesh and yet it can contemplate itself contemplating the meaning of life, asking questions. Questions like; Who are we? Where have we all come from? What was it that made us this way? How this Universe began? Why is it expanding at an accelerating speed? How is it likely to end? Are we alone in the entire Universe or there are other creatures who live in deep dark space of this vast Universe who may or may not look like us?

Our Brain is a three-pound flesh. It is made of 86 billion neurons. Each neuron is linked to other neuron by 10,000 to 100,000 connections called Synapses. This network of neurons forms an information superhighway through which flows information from our brain to every single cell of our body. The total number of Synapses, their combination and their permutations exceed the number of visible stars at night sky. Millions of Synapses join to form Neuronal Circuit. Neuronal circuits serve as information superhighway through which information flows from brain to all parts of our body. That is where our memory is stored. Our memory connects our past present to our future. Through our five senses, we receive a billion bits of data each day. When we sleep, our Brain process the information. A small fraction of the information is retained in the Hippocampus and Cerebral Cortex of our Brain, which is the library of our language and our Consciousness. The rest of the information is discarded. The retained information is restored, retrieved, cut, and paste and process faster than any computer. All the information is stored in Neuronal circuits and Cerebral Cortex of our Brain. Neuronal Circuits connects every

neuron with every other neuron forming a Wiring Diagram linking the entire Brain. Millions of Neuronal Circuits interact to generate our thoughts and our ideas and our visions. The complexity of our Brain is the result of three and a half billion years of Biological Evolution. It is a perfect organ in the Universe. It is a seat of our consciousness.

One day something terrible happens to our Brain. A single molecule of a single nucleus of a single neuron is damaged by radiation, chemical/environmental pollution or Viral infection, or genetic inheritance, the whole Brain collapse like a house of card, it becomes non-functional. A single normal cell becomes abnormal leading to cancer forming a tumor called Glioblastoma, one of the deadliest forms of Brain cancers. Brain Cancer is very different from Liver or Lung cancer. For example, if a Liver cell is similar damaged by radiations or chemical/environmental pollutants. The damaged Liver cell will mutate, divide, multiply, replicate, differentiate, metastasize, invade, and spread, shutting genes after genes and organ after organ killing the patient. It takes years, but not Brain tumor. Glioblastoma is a solid and aggressive tumor. It grows so rapidly within months it becomes so large. Its sheer size will crush the synapses, crush the neuronal circuits, and crush the wiring diagram and most patients will internally bleed to death within fourteen months. One of the greatest challenges of the nanotechnology is to seek out the very first abnormal cell and shut off the gene before it spread.

My assignment at NCI was to translate animal work to human. I was inspired by a speaker. During a drug delivery seminar at NIH, a speaker presented a paper in which he stated that he could identify the location of any drug in mice by injecting C-14 radiolabeled methylated compound of most drug and by taking X-ray within 24 hours. As an example, he methylated Quinone by using C-14 radiolabeled diazomethane in sodium hydroxide. When C-14 tetra methyl quinone was injected in mice, within 24-hour, X-ray showed the presence of radioactivity in the brain. Blood Brain Barrier (BBB) prevents most drug going to the brain except narcotics. Quinone is the first non-narcotic compound crossing the BBB. I immediately realized that I could selectively chlorinate quinone molecule and substitute one chlorine at a time with either Aziridine or Carbamate to attack Glioblastoma multiforme, the brain tumor in humans, which is a solid aggressive tumor like Walker Carcinoma in Rats. I decided to use Quinone moiety as a carrier for Aziridine rings to attack Glioblastomas. By introducing an additional Carbamate moiety, I could increase its toxicity several folds. I planned to use this rational to translate animal work to human by introducing multiple Aziridine and Carbamate moieties to the Quinone to test against Glioblastomas in humans. Attaching two Aziridines and two Carbamate moieties to Quinone, I synthesized Di-Aziridine, di-Carbamate Quinone. I named this novel compound AZQ. By treating brain cancer with AZQ, we observed that Glioblastoma tumor not only stop growing, but also start shrinking. I could take care of at least one form of deadliest old age cancers that is Glioblastomas. Literature search showed that AZQ is extensively studied [17].

I worked on this assignment about a quarter of a century; conducted over 500 experiments which resulted in 200 novel drugs. They were all tested against experimental animal tumors. Forty-five of them were considered valuable enough to be patented by the US Government (US Patent 4, 146, 622 & 4,233,215) [18, 19]. One of them is AZQ which not only stop the growth of Glioblastoma, but also the tumor start shrinking. For the discovery of AZQ, I was honored with, "The 2004 NIH Scientific Achievement Award." One of America's highest Award in Medicine. Complete lectures are available at https://www.facebook.com/ hameed.khan.7773/notes.

Figure 1: 2004 NIH Scientific Achievement Award presented to Dr. Hameed Khanby Dr. Elias Zerhouni, the Director of NIH during the NIH/APAO Award Ceremony held on December3, 2004.

Dr. Khan is the Discoverer of AZQ (US Patent 4,146,622 & 4,233,215), a Novel Experimental Drug Specifically Designed to shut off a Gene that causes Brain Cancer for which he receives a 17-year Royalty for his invention (License Number L-0I9-0I/0). To this date, more than 300 research papers have been published on AZQ. The award ceremony was broadcast live worldwide by the Voice of America (VOA). Dr. Khan is the first Indian to receive one of America's highest awards in Medicine.

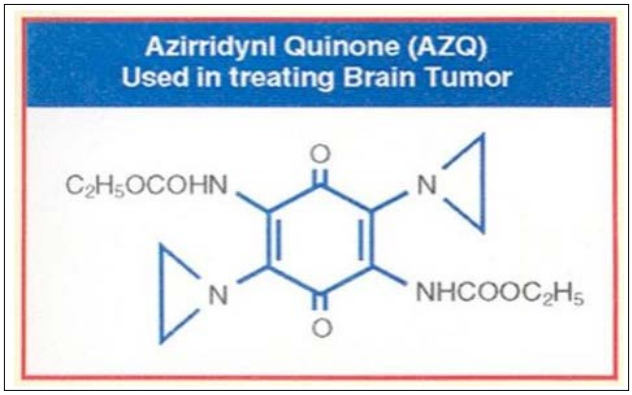

Figure 2

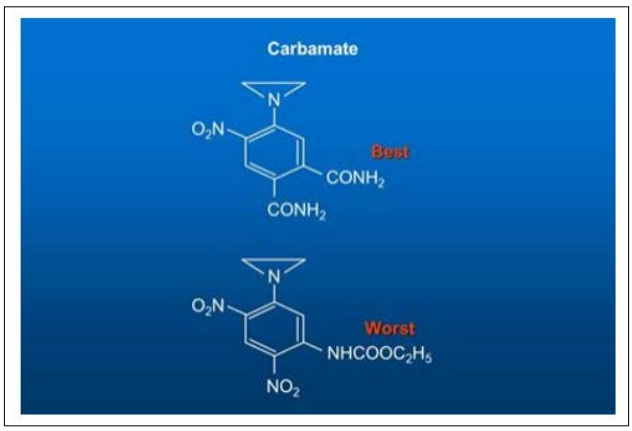

Figure 3: Single Strand DNA Binding Aziridine and Carbamate

Figure 4: Gold Medal for Dr. Khan. U.S. Patent 4,146,622

Figure 5: His Excellency Dr. A.P.J Abdul Kalam, The President of India Greeting Dr. Hameed Khan Discoverer of anti-cancer AZQ, after receiving 2004, Vaidya Ratna, The Gold Medal, One of India's Highest Awards in Medicine at The Rashtrapathi Bhavan (Presidential Palace), in Delhi,India, During a Reception held on April 2, 2004.

On the Rational Ground, What Other Cancers Should We Explore Next?

Could I use the same rational drug design and introduce a novel method for treating Breast tumor? Although mutations on BRCA1 gene located on Chromosome-17 (which is made of 92 million nucleotide bases carrying 1,394 genes) has been identified years ago responsible for causing Breast Cancer, we wonder why it has been so difficult to design drugs on rational basis to treat Breast Cancer. By the time the Breast Cancer diagnosis is confirmed in a patient, the BRCA1 has accumulated more than three thousand mutations. Genotyping of the blood would also show the existence of many cells carrying mutated cells responsible for creating secondary deposits. It is also believed that by the time Breast Cancer is confirmed, metastatic cancer cells have already been spread from liver lung on its way to brain. Since all other organs including breast and liver could be removed and replaced by organ transplant except brain, I thought that protecting brain is utmost important to save life. Once AZQ is developed to protect the brain, I could focus on the Breast and Prostate Cancers. Recent, Radiolabeled studies showed that male hormone Testosterone has great affinity for female organs like Breast, Ovary, and Fallopian tube cells. On the other hand, Estrogen, the female hormone, has great affinity for the male prostate gland. By attaching multiple Aziridine rings and Carbamate ions to both Hormones, I could design novel drugs to attack the Breast and the Prostate cancer. Now, I found that I could increase its toxicity even further by attaching more than four Aziridine and Carbamate moieties to both Male and Female Hormones.

In a Breast tumor, within the start and stop codon, BRCA1 gene has captured over two hundred thousand nucleotide bases. The BRCA1 genes carries about three thousand mutations. These mutations are caused by exposure to radiations, chemical or environmental pollutants, viral infection, or genetic inheritance. To attack the mutated nucleotides among the three thousand mutations in BRCA1 gene, we could use male hormone, Testosterone, and bind multiple radio labeled Aziridine and Carbamate ions to attack BRCA1 mutations. By using three dimensional MRI, we could show how many radio-labeled nucleotides were bound to which mutations. Out of seventeen positions available for substitutions on Testosterone ring system. There are only three active positions that is 1,3 and 17 are available for substitution on Testosterone ring system. Carl Djerassi had demonstrated that we could activate position 9 and 10 by reacting with Bromo-acetamide which introduce a Bromo ion on position 10 which could be de-brominated by Collidine to introduce a 9,10 double bond which we could further brominate to produce 9,10 dibromo compound [20,21]. These bromo ion could be replaced and activate 9,10 position for substitution and introduce additional Aziridines or Carbamate ions. We could increase or decrease the number of Aziridine and Carbamate ions to get the maximum benefit by further brominating position 15 and 16 to introduce additional Aziridine and Carbamate moieties.

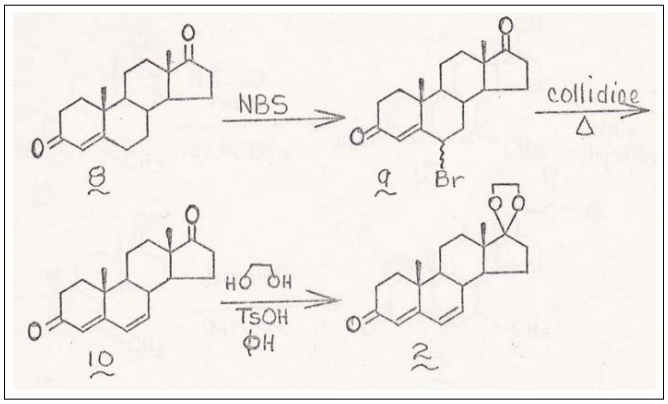

Figure 6

Similarly, we could use the female hormone Estrogen and by attaching multiple Aziridine and Carbamate ions to attack Prostate tumor. Since there are seventeen positions also available on Estrogen ring as well; again, we could activate additional positions for substitution and increase or decrease the number of Aziridine and Carbamate ions to get the maximum benefit.

Cardiovascular Diseases

Coronary Artery Disease is complex involving about 60 genomic variants (genes). All variants are not clustered on any specific Chromosome. These variants are dispersed across the entire genome. Although all variants have not been sequenced, we can shut off only the mutated gene without knowing the sequence of all other genes. As I mentioned above in the Cancer Section, the mutated gene grows rapidly forming the tumor. As it grows, it uses Glucose as a source of energy which is broken down to produce Lactic Acid. In the presence of Acid, the analogs of Aziridine and Carbamate are activated to generate Carbonium ion which attack the tumor DNA shutting off their genes.

While we may someday be able to sequence all 60 genes associated with the coronary artery disease, presently, we can single out and identify the mutated gene bound complex using radiolabeled Aziridine and Carbamate. The following example explain how some Arrhythmias causing genes could be identified and how drug could be designed to shut off these genes.

The term "QT" refers to the segment of an electrocardiogram which measures the duration of time for the heart to relax after a heartbeat. In long QT syndrome, the duration of time is abnormally prolonged and creates a vulnerability to dangerous arrhythmias [22]. Ever since the syndrome was described in 1957, researchers have engaged in a genetic race to identify the genes associated with long QT syndrome, which currently includes 17 genes. Three genes, KCNQ1, KCNH2 and SCN5A, had sufficient evidence to be implicated as "definitive" genetic causes for typical long QT syndrome. Four other genes had strong or definitive evidence supporting their role in causing atypical forms of long QT syndrome, presenting in the newborn symptoms associated with heart block, seizures, or delays in development. Once the mutated genes are identified, we could design drugs to shut off these genes as described in the Cancer Section.

Alzheimer

In 1906, the German Physician Scientist Dr. Alois Alzheimer identified the microscopic changes in the brain of a patient with the memory loss. He was the first Physician to identify the disease in a fifty-year old woman who suffered from Psychosis and who died within 4 years. Using special dyes, he stained the brain tissues which carried abnormal protein deposit around her brain which controlled brain function. He identified two kinds of legions of amyloid patches which he mistakenly thought was fatty patches and now turned out to be proteins. He observed a Patch of fatty deposit on the top of the brain cells called Plaques and the legions inside the nerve cells called Tangles. He accurately correlated the abnormal protein deposits around brain cells which controlled of brain function [23-26].

Today, we know that the Age is the single most risk factor for developing Alzheimer. By age 65 or older, the risk for developing Alzheimer is about 10 percent and by age 85 or older the risk factor is as high as 40 or 50 percent. As people grow old, they become senile. When he performed the autopsy of many senile persons, Dr. Alzheimer found the same Plaques and Tangles in many other samples. Early onset or late onset of Alzheimer resulted in the epidemic of Alzheimer. When comparing a normal brain with the Alzheimer brain, we find that the Alzheimer brain has shrunken and there is a concentration of Plaques and Tangles in neurons. In healthy brain cells, we see occasional Plaques and Tangles. It defines the disease; the Plaque and Tangles start building up as we grow old and over years and decades, the symptoms begin to develop. Symptoms include Memory loss and decrease ability of learning and recall. Early onset affects cognition which encompasses memory and other mental functions such as erosion of attention, thinking, reasoning, visual functions, spatial function, and Dementia with Memory loss and other cognitive functions resulting in mental impairment which affects to the degree interfering with the daily life.

Recent studies confirm that Alzheimer is an irreversible brain disorder which slowly destroys memory and thinking skills. The damage to the brain is not particularly associated to any specific gene, but the presence of the one form of the Apolipoprotein E (APOE) is a suspect gene whose presence does increase a patient's risk for developing Alzheimer. The early onset of Alzheimer is associated with three single gene mutations: First, the presence of an Amyloid Precursor Protein (APP) located on Chromosome-21; the presence of Presenilin 1 (PSEN1) on Chromosome-14 and the presence of Presenilin 2 (PSEN2) located on Chromosome-1. All three Chromosomes are very large and carry hundreds of genes. For example, Chromosome-1 is the largest Chromosome in the Genome. It is made of 163 million nucleotide bases carrying 2,610 genes. Chromosome-21 is made of 50 million nucleotide bases carrying 337 genes while Chromosome-14 is made of 109 million nucleotides bases carrying 1,173 genes.

A recent 7-million Utah population study identified two additional genes RAB10 located on Chromosome-2 (which is made of 155 million nucleotides bases and carry 1,798 genes) and SAR1A gene located on Chromosome-10 (which is made of 144 million nucleotide bases and carry 983 genes) associated with the formation of Plaques and Tangles. Mutations on these genes may be associated with the onset of Alzheimer. Of all the genes on these Chromosomes, only five single-gene mutations are associated with the early onset of the Alzheimer, it is the greatest challenge to design drugs to attack only the mutated genes. As I said above in the Cancer Section, the good news is that the only mutated genes grow rapidly using Glucose as a source of energy. Glucose is broken down to produce Lactic Acid. It is the acid which activate the Aziridine and Carbamate moieties producing powerful Carbonium ion which attack N-7 Guanine of DNA and shut off only the mutated genes. Other genes are not affected. Using C-14 radiolabeled Aziridines, we can identify the mutated gene which form the Aziridine/Protein Complex as described in the Cancer Section.

Rationale for Designing Drugs to Treat Alzheimer

It is well known that using the TFT dye, which is (3,6-dimethyl-2-(4-dimethlaminophenyl)-benzothiazoline, could be used to stain the Plaques and Tangles of Alzheimer tissues. Using TFT dye as a carrier for the Aziridine and Carbamate moieties, we could design prodrugs to attack the mutated DNA to shut off genes which form Plaques and Tangles to prevent the progress of Alzheimer.

In the above Cancer section, I have described in detail how I had used Quinone as a carrier for Aziridine and Carbamate ions in designing AZQ to attack the brain tumor DNA to shut off genes for treating Brain Cancer. Similarly, the analogs of Benzothiazoline dyes could be used to carry Aziridine and Carbamate moieties to attack the Plaque and Tangle DNA and to shut off genes responsible for causing Alzheimer.

Robotic Exploration of Exoplanets

We lack the ability to travel long distance to find a second Earth, but we could send robots in place of humans. James Webb Space Telescope is the most complicated telescope, three times the size of Hubble Space Telescope. It will not only detect new planets and signs of life in exoplanets but also it will detect signature of life supporting gases. It will serve as a life finder; looking for biological signatures such as photosynthesis by vegetations, using spacecraft like Voyager I, can we look back and see our planet's atmospheric condition and searching for the signature of life. Instead of risking the life of astronauts. We could send Robots to study the atmospheric composition by spectral analysis for the presence water of the nearest exoplanets.

Like US Human Genome Project, the Europeans have embarked on the Human Brain Project. When completed, Robots will carry

the artificial brain for deep space travel to make early assessments for human survival of the exoplanets.

The Human Brain Project

While America completed Human Genome Project, The Europeans have approved the Human Brain Project. In The Human Brain Project, every neuron will be transistorized, and every neuronal circuit will be converted to electronic circuit and will be saved in the computer as a normal human brain saves the information. Our Brain is a three-pound flesh. It carries 86 billion neurons. Our synthetic Brain will carry the total information in 86 billion neuronal circuits which will be converted to 86 billion electronic circuits.

Constructing one electronic circuit at a time, we hope to transcribe total information from the Organic Human Brain to the Inorganic (Computer CPU) Electronic Brain. When completed, we expect the Human Brain Project to search the computer's encyclopedia and answer all our questions with the speed of light. Recently, the Swiss group received one billion Euro from the European Union to proceed to complete the Human Brain Project. Although one billion Euro seems large amount, the complexity of the Brain will require at least one hundred times additional money. For example, to partially stimulate Human Brain, (to stimulate less than five percent of the Human Brain's neurons and synapses) scientists at IBM expect to use 880,000 microprocessors which will not even be ready by 2030. When completed, the computerized miniature human brain, we will send robots for the deep space exploration in search of exoplanets. While self-sustaining city-sized Mother ship carrying space travelers will be orbiting the exoplanet, the unmanned daughter ship carrying the Robots with computerized mini brain will land on the surface of exoplanet studying the environmental conditions suitable of human landing. Today, Robotic technology is so advanced, we can assemble an army of Robots with programs of different skills to perform different function on the surface of exoplanet. We are waiting for the Human Brain Project to be completed. If not tomorrow may be day after tomorrow, once the brainy Robots start walking on the surface of the exoplanet, we can say that Humans are prized creation, and we are super special which is taken straight out of the Book of Genesis.

Before embarking on human flight to distant star systems, we must think of creating live microbial astronauts who would travel in deep space to survive under harsh conditions. They will carry special genes to survive in harsh space conditions, such as genes resistant to radiations, extreme hot and cold conditions and extreme desiccations and freeze-dried condition. They would pave way for humans to travel under harsh conditions.

Robotic Exploration of Water Worlds

Liquid water is essential for life to evolve, survive and thrive. Whether the water is on planets like Earth or Moons like Europa or Enceladus or Comets which are circulating mud balls. Besides drinking, water provides Oxygen for breathing and Hydrogen as a fuel. Early life thrived during Pre-Cambrians Era; only single cellular life survived in the frozen world. As the first sunshine appeared on early Earth, ice melted, life evolved. Single cellular living creatures attacked each other and become multicellular living creatures.

About twenty years ago, we have sequenced the entire Human Genome. Now, we are perfecting High Throughput sequencers to bring down the cost of sequencing to a thousand dollars per genome. We are developing next generation sequencers like Parallel or Nanopore sequencers that will decrease the cost of sequencing down to a few hundred dollars per Genome. By the turn of the century, all space travelers would have their genome done and inscribed on a computer chip the size of a penny which they will carry with them at the back of the wrest watch. In case of medical emergency, we could compare their genomes and help them instantly.

The Spectral analysis of the atmosphere of our planet Earth taken by Voyager I spacecraft from over 4 billion miles from Earth could provide a method to analyze deflected light from Earth during Sun's eclipse. The spectral analysis of the atmospheric gases on Earth will provide composition data that could be used to compare the presence of life-giving gases on our planets with the exoplanets on new star systems. The accuracy of the methods showing the composition of the Earth's atmosphere could be used to compare the spectral lines of atmospheric composition such as the presence of Water, Hydrogen, Hydroxyl ions, Hydrogen peroxide or Ozone on the newly discovered Earth like planets in distant star systems to see if they could support life.

For example, recent photographs (and spectra) taken by the spacecraft Cassini showed the presence of Hydrothermal vents spewing water vapors and ice particles from an underground ocean beneath the icy crust on Saturn's sixth largest moon, the Enceladus. If NASA were to send an unmanned spacecraft to collect the samples from these geysers, we have the technology to detect the presence of part per trillion biomolecules in these samples. While working for FDA (US Food & Drug Administration), I developed methods to detect the antibiotic residues in PPT (part per trillion) range using Californium-252 Plasma Desorption Mass Spectrometry of Aminoglycoside Antibiotics [27]. We could use the same method to analyze samples brought from Enceladus. Are the geysers made of steam hot water or they are frozen gaseous in nature? NASA is planning to land a robot near this site hoping to collect sample and bring back to Earth for analysis. What if we don't find any living microbe? Instead, should we come back empty hand or should we send microbial life to accelerate evolution. If we don't find life there, then we should take living molecules with us.

Our first aim should be to spread any life on our Solar System first. With our Robotic payload, should we send genetically modified Blue Green Algae which has been spliced with extremophile genes which make living cells resistant to extreme temperature, freeze-dried condition, and desiccation; it should also carry Chloroplast genes which absorb Carbon dioxide and release Oxygen in the atmosphere. Before landing, the Robot will circle around the Enceladus spreading on its vast surface spores of genetically modified Blue Green Algae before landing. The same Blue Green Algae brought the biological evolution on early Earth. It replicated living cells on the surface of frozen Earth three and a half billion years ago. We could genetically modify Blue Green Algae within days it could be spread on the surface of the Enceladus. If liquid water exists on its surface, Blue Green Algae will thrive. Over millennia, it could alter its atmosphere.

First, we have developed incredible ability to detect part per trillion quantities of organic material obtained either from our own planet or sample obtained from exoplanets. Second, from a single crystal, we can obtain its mass spectrum to determine its molecular weight and the fragmentation patterns. From a single crystal, we can obtain its X-ray crystallographic diffraction pattern to determine

its three-dimensional structure. Once its structure is determined, we can synthesize large quantity and find its usefulness.

If robotic missions bring samples to Earth from distant star systems, we have the technologies to analyze and identify the samples in part per trillion ranges. The man who developed these technologies was at NIH. He was Dr. Henry M. Fales one of the greatest Americans scientists, I came across. He was the Head of Mass Spectrometry Department at NIH in Building 10 and used to work on the 7th Floor of the Clinical Center. I came to work for him as his Post-doctoral Fellow in one of his several Labs. He used to teach Mass Spectral analysis in the evening courses. I was his students as well. During Nixon Administration, he was invited to join a team of scientists on a ship called Alpha Helix going to the heart of Amazon Rain Forest basin for collecting samples to detect unusual life forms for identification and analysis. The novel samples were purified and analyzed by Mass Spectrometry. The Mass Spectral fragments were identified, and various fragments were put together to create the original molecule. If the new molecules are beneficial to humans, we could make large quantity for general use, but if it is harmful, we could design drugs to shut off genes responsible for causing harms.

Working with Dr. Helen Llyod in one of the many laboratories of Dr. Fales' Mass Spectrometry Department, I had isolated, analyzed, identified, and synthesized 8-Hydroxyisocoumarin and its analogs from an unusual beetle brought from Amazon Rain Forest [28]. The idea is if using mass spectral analysis, we could identify samples from our planet Earth; we could also analyze and identify biological samples from exoplanets in part per billion range if their genetic sequences are read by robots beam back to Earth. Once it reads the Genetic code, the robot could convert to Digital Code and beam its sequence data back to Earth with the speed of Light. We could receive the Digital Code and re-convert to its Genetic Code and read its sequence. Using the nucleotides from the Lab, we could recreate the structure and determine what kind of life exist on the exoplanet. With this knowledge, we could send future spacecrafts equipped with sequencer/converter to any one of those recently discovered 4,000 exoplanets in the Milky Way Galaxy to find a habitable planet for humanity.

NIH has all the facilities to analyze any exoplanet samples brought by Robots. Dr. Fales used to say today samples are available from various part of our planet; tomorrow you will get samples from exoplanets. The method of analysis would be the same. If the Life on exoplanets is made of DNA and have evolved from the same Carbon-based life forms, then they would also be made of the same four nucleotides, Adenine (A), Thiamine (T), Guanine (G) and Cytosine (C). You would have real challenge, if the life on exoplanet is evolved from the Silicone based life forms.

As a part of the FDA's harmful residue detection program in the tissues of the food producing animals, using "Californium-252 Plasma Desorption Mass Spectrometry of Aminoglycoside Antibiotics," we demonstrated the presence of part per trillion antibiotics in the tissue extracts from the edible tissues [27]. We could use the same technology to detect the presence of any life form if brought from the Saturnian Moon Enceladus geysers' samples.

We have similar geysers on Earth. For example, the geyser "Old Faithful," in the Yellowstone National Park in Wyoming, erupts every 91 minutes and reaches a height of 145 feet within 15 to 20 second. The water temperature at the vent has been measured at 95.6 degree Centigrade. At this high temperature, most microbial life forms die. The enzyme DNA polymerase essential for growth breaks down. Heat resistant microbial life-forms evolve over millennia do survive at this high temperature. A gene that produces heat resistant DNA polymerase enzyme called Taq DNA polymerase has been isolated from thermopiles. Taq DNA polymerase is extremely useful. During DNA enhancement by PCR (polymerase Chain Reaction), when repeated heating and cooling of DNA is required for doubling the amount of DNA, the heat resistant Taq DNA polymerase is added only once for repeated doubling of DNA.

Samples from Enceladus brought by the robots could be analyzed for the presence of Taq DNA polymerase or we could send a miniaturized DNA-sequencer (which determines the number, and the order of the nucleotides bases in the molecules) on the unmanned spacecraft to sequence the presence of Taq DNA polymerase and send its spectral data to Earth for its identification.

Are We Alone in The Universe?

The answer is that the Universe is vast. It must be teeming with life; the distances are so vast, that we have no means of communicating with them. Now, we are looking for two sets of evidence. First, if life exists on distant star systems, how advance are they? Are they communicating with one another using radio technology? Could we detect their means of communications by radio wave signals? Through our TV and Radio, we have been broadcasting and sending radio signals for over a hundred year which should have covered a hundred light year distance in every direction. If there is an advanced civilization within hundred light year radius, they should have received our radio signals. We have not received any response signal so far. It does not mean that no living creature exists within a hundred light year distance. Microbial life forms may exist. Second, we are looking for spectral lines for signature gases such as Oxygen, Carbon dioxide, Methane, Nitrogen, Water vapors, Hydroxyl ions which prove the existence of some primitive forms of life at some distant star systems.

As I said above, the most logical prediction was provided by Dr. Frank Drake. In 1961, Dr. Frank Drake, a radio astronomer, working at the National Radio Astronomy Observatory in Green Bank, West Virginia, estimated the probable number of technological civilizations that might exist among stars. Now, known as the Drake Equation which roughly estimates what is the likelihood that we would be contacted by an extra intelligent life beyond Earth. How often that would happen? Drake equation attempts to Search for Extra Terrestrial Intelligence (SETI) in the Universe and encapsulate the critical considerations necessary for predicting alien life in other Star Systems and how often they will be contacting us.

First, we need to know the rate at which the stars are produced at the center of a patch of star systems in which intelligent life might live. Then we multiply by number of stars that have planets. Then we multiply by those planets which develop life which is then multiplied by the planets which develop intelligent life that have developed technological communication skills detectable by radio signals. Although the equation predicts the existence of millions of such civilizations in our Milky Way Galaxy alone, the equation contains several factors which are highly conjectural and their combined effect being that the uncertainty associated with any derived value is so large that the equation cannot be used to draw firm conclusion. The equation is an attempt to inspire scientist to take these factors into consideration when contemplating the question of other radio-communicative methods used by intelligent life. Since the inception of SETI, we have not received a single reproducible

radio signal from anywhere. Through our TV and radio broadcast, we have been sending radio signals for more than a hundred years. These signals travel with the speed of light. By now, our radio signals have reached more than a hundred light years in every direction. Since we have not received any signal from anywhere, we assume that there is no technically advanced civilization exists within a hundred light year distance. In future, we plan to send all radio signals by cables; our world will go radio quiet. It gives the impression to aliens that technically advanced civilizations like our have destroyed ourselves by going to Nuclear War.

Under similar conditions as on Earth, could life evolve on other Star Systems? Universe is vast. In our own Milky Way Galaxy, there are over one hundred billion Star Systems like ours. Every star is like our Sun. All stars have planets. Every star in the Milky Way Galaxy should have at least one planet. Jupiter size planets are very rare. As I said above, closer planets to the Stars have high temperature. Hot enough to melt rocks. Distant planets from the stars are frozen. Small planets are extremely common. If we are looking for life beyond our Solar System, it may not be like ours. The Greenhouse gasses in the atmosphere make the planets hotter or colder. If we are searching for life on distant planets, we should look for Planets in the habitable zone which are not too hot not to cold just right for life and we want to look for the atmospheric gases. Light reflected from the planets could be analyzed by spectral method to determine the presence of Greenhouses gases.

The James Webb Space Telescope is scheduled for launch in early November/December 2021. A large space telescope optimized for infrared wavelengths, the Webb telescope will find the first galaxies that formed in the early universe and peer through dusty clouds to see stars forming planetary systems. The Webb is the successor to Hubble, and it's 100 times more powerful. Webb also has a much bigger mirror than Hubble, explains the Webb telescope site: "This larger light-collecting area means that Webb can peer farther back in time than Hubble is capable of doing.

While NASA is working on the Fusion Reaction, attempting to produce unlimited amount of clean energy from the sub-atomic particle, preliminary results indicate that it is possible to create Fusion reaction in the Lab and generate unlimited amount of clean energy like Sun where Hydrogen atoms under extreme pressure and temperature fused to form Helium and produces subatomic particles photons which come to Earth as Sunlight.

We at NIH could contribute to Computer Revolution as we need to replace Silicone Chips which melts at high temperature. We need to make computer chips of Graphite, Diamond or DNA which will store exponential amount of information for centuries and which is stable at higher temperatures. Our answer to the third question is that our destiny lies outside Earth. We must escape our Solar System. If intelligent life extinguishes from the Earth, no one will ever know if we ever existed. Our Prime responsibility is not only to protect, preserve and spread human intelligence in every corner of the Universe, but also to keep humanity alive. We must find a second Earth, with similar life supporting ingredients on any planet in different star systems. To send intelligent life on a one-way journey for the search of Second Earth, we must train an army of scientists mastering in Genetics, Robotics and Nanotechnology.

As I said above, Mother Nature is too slow for living creatures to evolve from microbes to mankind. Within a billion year after our solar system was formed, first living microbes, the blue green algae, was evolved, but the intelligent life was evolved only three million years ago when the Sun has used up more than half of its energy during the four and a half billion years. We will accelerate the evolutionary process. The second genesis will not start from microbe to mankind. From the IVF experiments, we learned that the fertilized ovum could be kept alive intact for centuries in liquid Nitrogen below seventy degrees centigrade. We could take millions of fertilized eggs with us to colonize new star systems.

On March 7, 2009, NASA launched the spacecraft Kepler. It is a space observatory whose function is to discover the Earth size terrestrial planets revolving in the habitable zone of billions of stars in our region of the Milky Way Galaxy. By the time Kepler's four-year mission ended in May 2013, Kepler observatory has found 4,000 exoplanets in about 440-star systems. Is there life in one of those exoplanets? We have no proof of the existence of life beyond Earth.

Kepler Space Telescope focused on a patch of sky crowded with stars. It made remarkable discoveries. For example, Kepler 186X, is a star system with five planets and Kepler 186F is in the habitable zone. Kepler scientists claimed that there are at least 5,000-star systems like ours. They also claimed that Kepler 90i is most likely to have Earth like atmosphere. Is there any form of life on any of those Star Systems? We have no proof of their existence.

Our Sun is a star. It carries eight planets and 140 moons and millions of asteroids and comets revolving around our Sun forming our Solar System. There are over 100-billion-star systems in our Milky Way Galaxy alone. There are about 4 hundred billion galaxies in the visible part of the Universe.

During eclipse, Star light is completely blocked by the planet called the Star Shade. Once the star light is blocked, the star light shines the neighboring planets which are invisible when the star light is not blocked. Light deflected from distant planets could be analyzed by modern spectrometers. The presence of spectral lines for Oxygen, Water vapors, Carbon dioxide, Methane, Ozone etc. identify the existence of life. Because Oxygen is the byproduct of photosynthesis, its presence confirms the presence of plant life. The next step is to identify the presence of other Greenhouse gases such as Carbon dioxide, Methane, Hydroxyl ions, Ozone and Nitrogen.

As I said above, the more powerful next generation telescope called the James Webb Space Telescope will be launched in November/December 2021 whose function is to study the history of evolution of our Universe from its beginning as a luminous glow after the Big Bang to the present formation of billions of Star Systems including our own. If you would move your TV dial between two channels, the snow effect on your blank TV screen is the result of millions of photons left over from the Big Bang.

After his speech, senior scientists thought that Dr. Goldin's ideas were the craziest that they had ever heard. Junior scientists were mesmerized, and they thought his was the most inspiring speech. About twenty-one thousand scientists work at NIH. NIH is made of 50 buildings; building 10, the Clinical Center; is the largest brick building in the world. It has hundreds of labs in which thousands of scientist workday and night where they study hundreds of patients on clinical trials. It has 17 miles long underground tunnels carrying goods and services to various labs. Imagine the future spaceship made of Graphene ten times the size of Clinical Center. Let us call CC-I which is constructed 33 miles above the Earth surface

where there is no air. For future travelers, a fleet of spaceships say from CC-I to CC-100 could be built with Graphene. It is thinner than a paper sheet and 200 times stronger than steel and incredibly flexible. It is ultra-light yet immensely tough. It is the thinnest material possible as well as being transparent. It is a superb conductor and can act as a perfect barrier - not even Helium gas can pass through it. These Graphene manufactured spaceships could serve as micro-Earths; they would have the ability to recycle air, water and food and could survive independently without any assistant from Earth. They would be free to travel to any distant star systems looking for habitable planets. Within ten light year distance, 4,100 Exoplanets have been discovered so far. It is believed that more than a 100 billion habitable planets of varying orbits, sizes and compositions exist in our Milky Way Galaxy alone which is about 250,000 light years across. Is there life in any one of those exoplanets? We don't know, but spectral analysis showed that Life giving atmosphere do exist on those planets. For the past hundred years, we have been sending TV/Radio electromagnetic signals in every direction with the speed of light. For over sixty years, SETI has not received a single reproducible signal to confirm the existence of intelligent life within one hundred light year distance. Do we wait for another sixty years, or do we take action to search for an exoplanet suitable home for humanity? Now, we have learned to convert analog language of Biology to the Digital language of computer. We can send the analog language of Biology as electromagnetic signals as sequence information to the Digital Language of computer as digital-biological convertor to Mars to the next generation of human Marians with the speed of light. If they find a microbial fossil, they can send its sequence information to Earth where our sequencer/convertor will reconvert the digital language to DNA sequence and using the sequence data, wecanrecreate the creatureinour Labs.

On the other hand, if the human residents of Mars require the genetic sequence of any plants or animals, we can send the information with the speed of light. The only condition is that exoplanets' life form is also based on the DNA sequence using AT-GC nucleotide bases. What if the life in the exoplanets is based on Silicone instead of Carbon? We will have problem. We cannot speculate until we see some evidence of existence of life in Silicone.

We are neither special nor unique. The Universe began as a single mass of energy made of the same material. The basic element Hydrogen fused during Big Bang to form all 120 elements exist on Earth. Different galaxies are made of the same material of the original explosion. Based on this fact, it is reasonable to assume, if life evolved, it should be made of the same material. We can send unmanned spacecrafts to the nearest exoplanet equipped with sequencer/convertor to analyze and sequence any form of life either alive or in the fossil forms. There is no doubt that life evolved on Earth over billions of years ago. The earliest components that constitute life has been created in the Labs.

In 1953, Stanley Miller in Chicago University conducted a simple experiment. He mimicked the early atmospheric conditions that existed on Earth. In a round flask containing Ammonia, Carbon dioxide, Methane and Water, he sparked electric current for a week. It turned to a dark mixture. He analyzed the dark mixture and isolated amino acids the essential life-giving molecules. Based on the above experimental evidence, we hypothesized that the appearance of life is based upon the fact that since a million-lightning strike Earth each day, at some remote corner of the Earth, lightning struck at a cloud of gases containing Nitrogen, Carbon dioxide, Water vapors, at a rock containing Phosphate whose interaction over three and a half billion years resulted in the creation of the first information molecules called Nucleotides whose interactions give rise to a long string of letters called RNA (Ribonucleic Acid) consisting of four nucleotides Adenine (A), Thiamine (T), Guanine (G) and Cytosine (C). Nucleotide bases constituting RNA (Ribonucleic acid). Life on Earth began as an RNA World because RNA has the ability not only to store information like DNA, but also to catalyzed reaction like Proteins. All four nucleotides have also been synthesized in the Labs by Cyril Penumperuma of the University of Maryland and they all carry the same information and perform the same functions as natural DNA and RNA. Other more complex and stable molecules like DNA and Proteins were isolated later. Accumulation and rearrangements of these molecules over millions of years resulted in early microbial life on Earth. The early microbial life was trapped in the frozen Earth. No unicellular fossil is available. Fossils began to appear about 550 million years ago during a period called the pre-Cambrian Era when the first Sunrise appeared on Earth surface, the ice began to melt, and unicellular organisms attacked other unicellular organisms forming multi-cellular organisms. When frozen Earth became the Water World, the complex life evolved rapidly. The first early life must have been the Blue Green Algae. The essential elements such as Carbon dioxide, Methane, Nitrogen and Phosphorus were available for the Blue Green Algae to thrive. For the next billion years, Blue Green Algae must have carpeted all the available surface of planet Earth. Its job is to absorb Carbon dioxide and pump Oxygen. Today, we have 80 percent Nitrogen and about 20 percent Oxygen with trace amount of Carbon dioxide (400 PPM). Life thrived on Earth. Life evolved and nature selected. Nothing in biology makes sense except in the light of evolution, the tenet that all life has evolved and diversified from a common ancestor is the foundation from which we approach all questions in biology. Evolutionary science explains the unity of life by its history, whereby all species have arisen from common ancestors over the past 4 billion years.

Four and a half billion years of biological evolution resulted in us. All this information is trapped as fossil records in the layers of rocks. (All fossil records are on display in the Smithsonian Museum in Washington). Radio-active Carbon dating showed that the most ancient rocks have the simplest fossils, and the more recent rocks have the more complex fossils.

The Precambrian is the earliest of the geologic ages, which are marked by different layers of sedimentary rock. Laid down over millions of years, these rock layers contain a permanent record of the Earth's past, including the fossilized remains of plants and animals buried when the sediments were formed. The next step in the evolutionary process was finding the more advanced fossil. The Burgess Shale is a fossil-bearing deposit, found in a 505-million-year-old rock formation made of mud and clay exposed in the Canadian Rockies of British Columbia, located in Yoho National Park, Canada. It is famous for the exceptional preservation of the soft parts of its fossils. Trilobites are a group of extinct marine arthropods which represent the next step of evolution. Human fossils were found in a three and a half million old rocks found in the HaderValley in Ethiopia (Hader, site of paleoanthropological excavations in the lower Awash River valley in the Afar region of Ethiopia. It lies along the northernmost part of Africa's Eastern (Great) Rift Valley, about 185 miles (300 km) northeast of Addis Ababa) where Chimps were living for the last twenty-six million years. Recent DNA sequencing showed that Chimps shared 98.9 percent genes identical to humans. Just one point one percent genes in human's brain turned on to give us.

Today, there are more than seven and a half billion people live on planet Earth. We are adding 90 million newborn each year. By 2050, the human population is likely to be nine billion. We need to spread human intelligence on other Star Systems. So, we asked the most logical questions: Are we alone? Is there life out there? Who else is out there? Under similar early Earth conditions, could life have evolved on other Star Systems. Universe is vast; it must be teeming with life. Oxygen essential for life does not exist among Star Systems. Star Systems are separated by billions of miles of vacuum. If life exists, it must have been separated by billions of miles apart. How many Star Systems have evolved life like Earth?

There are three million known and thirty million unknown creatures live on planet Earth. We all have one thing in common. Our book of life, our Genome, is Carbon based and is written in the same language. From a tiny blade of grass to the mighty Elephant. And yet we are so diverse, no two individuals are alike. Even identical twin is not identical, they grow up to become two separate individuals. We cannot fly like birds or swim like fish, or run faster than Cheetah, see like Eagle or smell like Dog. What distinguish us from the rest of the creatures is that we think better than any creature on Earth. We carry Brain. It is neither bigger than the Brain of Elephant nor of a Blue Whale, but it is most complex. Our Brain is a three-pound flesh that you can hold in the palm of your hand. It can contemplate the vast distances among billions of Galaxies across Universe; It can contemplate the concept of Infinity. It convinces us to believe in existence or non-existence of God. It questions our Ethics; our Morality, our Altruism and our Free-will. It is only a three-pound flesh and yet it can contemplate itself contemplating the meaning of life, asking questions. Questions like; Who are we? Where have we all come from? What was it that made us this way? How this Universe began? Why is it expanding at an accelerating speed? How is it likely to end? Are we alone in the entire Universe or there are other creatures who live in deep dark space of this vast Universe who may or may not look like us? Our Brain is a three-pound flesh. It is made of 86 billion neurons. Each neuron is linked to other neuron by 10,000 to 100,000 connections called Synapses. Total number of Synapses, their combination and their permutations exceed the number of visible stars at night sky. Millions of Synapses join to form Neuronal Circuit, that is where our memory is stored. Our memory connects our past present to our future. Through our five senses, we receive a billion bits of data each day. When we sleep, our Brain process the information. A small fraction of the information is retained in Hippo campus and Cerebral Cortex of our Brain, which is the library of our language and our Consciousness. The rest of the information is discarded. The retained information is restored, retrieved, cut, and paste and process faster than any computer. All the information is stored in Neuronal circuits and Cerebral Cortex of our Brain. Neuronal Circuits connects every neuron with every other neuron forming a Wiring Diagram linking the entire Brain. Neuronal circuits serve as information superhighway through which flows information from Brain to every part of our body. Millions of Neuronal Circuits interact to generate our thoughts and our ideas and our visions. The complexity of our Brain is the result of three and a half billion years of Biological Evolution. It is a perfect organ in the Universe. It is a seat of our consciousness. It makes our Brain the supreme organ in the Universe. It is a kind of information processing unit, so far, our kind of intelligence is not to be found in the Universe. This is our prime responsibility to protect, preserve and spread our kind on intelligence in every part of the Universe.

Sharing Genetic Code with Alien Civilizations

During the past seventy years, since we broke the genetic code, from the student of learning genetic code, we became the master of genetic code, by cracking the genetic code to sequencing genetic code to reading genetic code, synthetizing the genetic code to converting genetic code to digital code. Now, we are ready to convert analog language of Biology to the Digital language of computers and transport our genetic code as digital code across the Universe with the speed of Light. We will be happy to share our knowledge with the Alien civilizations. We will share our entire human genome with them and tell them how to re-covert us from the digital code to genetic code and recreate us on their planet. We will share with them who we are where have we all come from. What was it that made us this way? We tell them that we are the result of three and a half billion years of biological evolution on Earth. They could re-create us in much shorter time. How intelligent creatures like us evolved on Earth? How our journey on Earth began as an RNA World to become the most stable DNA world. We tell them in detail how we are evolved so thatthe Alien Civilization couldcompare their ownevolution with ours.

We tell Alien Civilization that our logical thinking is not a hypothesis. It is the New World Order which is based on the experimental evidence, reproducible and verifiable results. The essence of life is information, and the information is located on DNA. Once the DNA is formed, evolution started forming the double stranded DNA. The biological information flows from DNA to RNA to Proteins. It is the double stranded DNA, which is transcribed into a single stranded of RNA (Ribonucleic Acid) and which is translated in Ribosomes to amino acids. The interaction of amino acids give rise to proteins whose interaction give rise to first living cells.

From the first cell to a complete human being, the biochemical detail is so similar that is highly likely that all organism from plants to animals on Earth are evolved from a single cell. This hypothesis, that all living creatures have a common ancestor, is supported by a variety of observations. For example, all organisms are composed of one or more cells. The organization and function of these cells have enormous similarities in their biochemistry such as their photosynthetic and respiratory apparatus. In the detail of their reproductive behavior of the cells. In the ubiquity of the molecule DNA as the genetic material, passing information from the parent's cell to daughter cells. In the detail of the metabolic breakdown of the food extracting energy.

In the symmetry of the constituent's molecules. Even in the microscopic detail of the membrane structure including the molecular basis of the color of their skin. Using the same material and the same method, all living cells are evolved repeatedly in extremely diverse aggregate to forms plants and animals which we describe collectively as Life.

Millions of cells interact to give tissues which interact to give plants. It is the plants which conduct Photosynthesis that is in the presence of water, Carbon dioxide and Sunlight, the genes (the specific collection of nucleotides) of the Chloroplast in plants convert Carbon dioxide to its food Carbohydrates and in this process, it releases Oxygen as its by-product. Accumulation of Oxygen over millions of years accelerated biological evolution leading to Natural Selection resulting in the appearance of aerobics life on Earth from microbe to plants to animals including humans.

We read the entire book of life (Genomes) of humans and many plant and animal species, letter by letter, word by word and sentence by sentence (sequencing) reading all essential ingredients (genes) in the books of their lives (Chromosomes). New generation of scientists are now sequencing the genomes of all living species on planet Earth. We have about three million known and 30 million unknown species on Earth. We have developed the toolkit of enzymes (scissors) to cut paste and copy and move around genes from one species to another. It is easier to grow plants than animals. We can make genetically rich plants which serve as factories to produce new food (by inserting essential amino acids genes), new fuel using Matheno-coccus found at the bottom of the ocean which converts Carbon dioxide to Methane, a fuel. The ocean surface around the world is releasing enormous amount of Methane in the atmosphere and new medicine (by inserting antibiotic genes) to treat every disease known to mankind to increase human lifespan beyond one hundred years.

Sequencing (Mapping) Plant Genome on The Production of New Food

Although there are many diverse and complex techniques involved in genetic engineering, its basic principles are reasonably simple. There are five major steps in the development of a genetically engineered crop. But for every step, it is very important to know the biochemical and physiological mechanisms of action, regulation of gene expression, and safety of the gene and the gene product to be utilized. Even before a genetically engineered crop is made available for commercial use, it must pass through rigorous safety and risk assessment procedures.

The first step is the extraction of DNA from the organism known to have the trait of interest. The second step is gene sequencing and cloning, which will isolate the pure gene of interest from the entire extracted DNA, followed by mass-production of the cloned gene in a host cell such as Yeast. Once it is cloned, the gene of interest is designed and packaged so that it can be controlled and properly expressed once inside the host plant. The modified gene will then be mass-produced in a host cell to make thousands of copies. When the gene package is ready, it can then be introduced into the cells of the plant being modified through a process called transformation. The most common methods used to introduce the gene package into plant cells include biolistic transformation (using a gene gun) or Agrobacterium-mediated transformation using Ti (Tumor Induced) Plasmid. For example, in the Genome of Ti-Plasmid spliced the codons of the essential amino acid and harvest in Yeast to make millions of clones or copies. Once the inserted gene is stable, inherited, and expressed in subsequent generations, then the plant is considered a transgenic. Backcross breeding is the final step in the genetic engineering process, where the transgenic crop is crossed with a variety that possesses important agronomic traits and selected to obtain high quality plants that express the inserted gene or codon in a desired manner. The length of time in developing transgenic plant depends upon the gene, crop species, available resources, and regulatory approval. It may take 6-15 years before a new transgenic hybrid is ready for commercial release.

We describe below to our Alien Civilization the ways of creating most nutritious vegetarian food without meat to feed the burgeoning population of the world. The new food would be non- toxic, non-allergenic, and their nutritional content would be comparable to their non-vegetarian counterpart. We will examine how vegetables can also serve as factories for producing modern medicine and finally, as the food supplies increases, the population also increases. We will examine the Ethical problem of population explosion.

To provide meat to vast majority of non-vegetarians, we need to raise millions of live stocks. All our daily function is performed by our body using 20 amino acids in different combination making proteins. Deficiency in any one of those amino acids cause our health problem. Out of 20 amino acids, our body does not make nine amino acids. We call them essential amino acids and they are: Valine, Leucine, Isoleucine, Phenylalanine, Tryptophan, Lysine, arginine, Histidine, Methionine, and Threonine. Although they are present in sufficient amount in the meat, but not in vegetables. We need outside source to introduce these amino acids in vegetables. They are present in some edible vegetables but are present in very low quantities. We eat meat to receive all amino acids. Producing vast quantities of meat is detrimental to our health and the health of our planet. First, eating meat has its own problem. In over-cooked meat, the amino acids decompose to produce N-Oxide a carcinogen. Second, to feed the world population, we must raise millions upon millions of farm animals. In cattle's stomach grasses decompose to generate Methane, a Greenhouse gas responsible for impacting Climate Change. Instead of increasing the number of farm animals, we must decrease animals to the lowest number, just enough to produce sufficient milk for the infants, and cheeses and Yogurts (curd) for adults. Instead of using animal milk, the adult population can use various vegetable juices like the Almond Milk in their breakfast cereals. The fruit juices are not only good for our health, but also good for our environments. They remove the excessive amount of Carbon dioxide from our atmosphere and reduce Greenhouse Effect.

In food producing animals, the cost of producing meat is very high. For example, to produce a pound of meat, we must feed meat-producing animals about a hundred pound of edible vegetables. We cannot waste edible vegetables to feed animals. As predicted by UN, by 2050, the World's population is likely to be nine billion. To feed nine billion, how many more food producing animals do we raised? The answer is a lot.

Now we have a new solution to our food problem. Since we broke the genetic code and unlocked the secrets of life, we are ready to manipulate life not only to clean up our environment pollution, but also to create new food, new fuel, and new medicine to treat every disease known to us on Earth.

After breaking the genetic code and unlocking the secrets of life, we are certain that through a process called Genetic Engineering, we could transfer the essential amino acids genes from the animals and insert into plants to enrich our vegetables diets with essential amino acids. The process of Genetic Engineering involves altering the genetic make of a plant using "Recombinant DNA Technology" which uses molecular scissors called the Restriction Enzymes to cut, paste and copy pieces of Codons which codes for the essential amino acids and transfer these codons from the animals to plants. Before reading the plant genome, that is reading its entire book of life, it is essential to read the Human Genome. To read the entire Human Genome is a colossal undertaking requires billions of dollars and years of effort of thousands of scientists from around the world. To read the Human Genome not only requires the funding from multi-national governments, but also requires the effort of thousands of scientists from six industrialized nations and 20 biomedical centers. This effort is led by US followed by Germany, France, England, China, and Japan. This was the greatest biological experiment ever conceived by Human mind. It

will answer the most fundamental questions, we asked ourselves since the dawn of human civilization. What does it mean to be human? What is the nature of our memory and conscientiousness? And our development from a single cell to a complete human being? The biochemical basis of our senses and the process of our aging? The scientific basis of our similarity and dissimilarity? Similarities that all living creatures from the tiny blades of grass to the mighty Elephants including man, mouse. Monkey and all plants from the plant kingdom are all made of the same chemical building blocks. And yet we are so diverse that no two individuals are alike. Even identical twins are not exactly they grow up to become two separate individuals.

In the nucleus of every cell, we inherit over 3 billion 2 hundred million genetic letters called nucleotides from our mothers and another set of over 3 billion 2 hundred millions nucleotide from our fathers forming nucleotide base-pairs. Less than two percent of our book of life carries instructions to make proteins called genes. The un-coded part of the genome carries switches, enhancers, promotors of genes. Healthy genes carry instructions to make normal proteins that maintain the healthy structure of our bodies. We still don't know the exact location of all 24,000 genes. We still don't know what different proteins those genes produce. We still don't know how those proteins functions individually and collectively in our body.

The double stranded DNA explained that the essence of life is information, and the information is located on these four nucleotides. Every set of three nucleotide on the mRNA forms a codon which codes for a specific amino acid. The four-letter text of nucleotides forms a three letter Codon which codes for an amino acid. There are 64 different combinations of Codons which codes for all 20 amino acids. Sequencing human genome identifies the number of nucleotides and the order in which they are arranged. Less than two percent of our genome contains regulatory region, a piece of DNA, which controls the function of genes. More than 300 regulatory regions have been identified. More than ninety eight percent of our Genome contains non-coding region used to be called the Junk DNA which makes up to sixty percent of our entire Genome. The non-coding regions contains repetitive piece of DNA which is tightly packed and mostly remain silent. The sequencing of this region showed that the non-coding region is the part of Viruses and Bacteria picked up by our Genome during the millions of years of our evolutionary process. During Bacterial or Viral infection, the non-coding DNA could unfold transcribing into RNA resulting into hazardous protein which could create havoc for our health.

For future space travelers, achieving Vegetarianism is the goal for humanity. As plant proteins are the primary sources of all dietary proteins consumed by human and animals and are inexpensive to produce in comparison with meat products; improving plant food quality will make a significant contribution to future needs of the population of the world. To provide most nutritious food to all of us, we must cut, paste and copy codons of all essential amino acids in edible plants or seeds. There are more than 4,000 molecular scissors called Restriction Enzymes have been isolated from Bacteria. There are more than 3,500 multi-functional Type II Restriction Enzymes which are commercially available to cut, paste and copy fragments of essential amino acid codons either into double stranded DNA, or into a single stranded RNA of various lengths.

Fortunately, there are more than one codon which codes for the same amino acid. If one codon does not transfer easily from animal to plant, we could use the other codons. About a quarter million of flowering plants exist on Earth today. We cultivate just about 150 plants species for Agriculture purposes. To feed over seven billion people of the World, we cultivate a mere nine species of these plants on large scale. They are Corn, Rice, Wheat, Barley, Sorghum/Millet, Potatoes, Tomatoes, Sugar Cane and Soybean. The other vegetables, fruits and nuts are cultivated in smaller amounts. The Genomes of most of these edible plants have been sequenced. Luckily, there are only nine essential amino acids. It would be most useful to splice these codons in their genomes to produce the most nutritious food. The world's population will get all essential amino acids without eating meat or large quantities of vegetables. Besides fruits and vegetables, there are three major plants eaten by most people of the world and they need our immediate attention, and they are Rice, Wheat and Corn.

Genes that carry essential amino acids are expressed in a two-step process and they are Transcription and Translation. First, the essential amino acids Codons are spliced or inserted into a double stranded of plant DNA which is later transcribed into a single stranded m-RNA. As I said above, it is the m-RNA which is translated in the Ribosomes into all 20 amino acids. The Cells decode m-RNA in groups of three nucleotides called Codons which carry instructions to produce the amino acids. When double stranded DNA is transcribed into a single stranded m-RNA, the nucleotide Thiamin is converted to Uracil. The Methyl group of Thiamine is replaced by a more water- soluble Hydroxyl group forming the Uracil. The nucleotide T for Thiamin is replaced by U for the Uracil. The m-RNA is translated into amino acids in Ribosomes. The gene expression has a Start Codon (AUG) which codes for amino acid Methionine and there are three Stop Codon which are UGG, UAG and UGA. Once the Stop Codon appears at the tail end of the DNA, amino acids synthesis stops. The Codons for each essential amino acid and their alternative codons are described below: Valine (GTT, GTC, GTA, GTG), Leucine (CTT, CTC, CTA, CTG; TTA, TTG), Isoleucine (ATT, ATC, ATA), Phenylalanine (TTT, TTC), Tryptophan (TGG), Lysine (AAA, AAG), arginine (CGT, CGC, CGA, CGG; AGA, AGG), Histidine (CAT, CAC), Methionine (ATG), Threonine (ACT, ACC, ACA, ACG).

Genes are the unit of inheritance. As I said above, out of four-letter text, that is A-T and G-C, and three letters code for an amino acid called the Codon. The starting Codon in a gene is the Codon AUG (instead of T nucleotide, we use U nucleotide because Thiamin is converted to more water-soluble Uracil) which codes for amino acid Methionine as I stated above. Long chain of DNA synthesis begins. The starting Codon is followed by a series of hundreds of Codons which codes for different amino acids in different species. There are three Stop Codons, and they are AUG, UGG, and UGA. Once the stop codons appear, DNA synthesis stops. Bacteria and Viruses have short codon chain. The longest chain is in a gene of Ducharme Muscular Dystrophy, a neurological disease whose chain extends to two and a half million codons. Once a gene is identified, using Restriction Enzymes (molecular scissors) like EcoR1, we can cut, paste, and copy all genes individually making a Restriction Site map. Once a single gene is isolated, we could compare the sequence of this gene with the Thousand Genome Project to identify differences called mutations which are responsible for the disease and design drugs to shut off that gene. Sequencing is like extracting Gold from its Ore.

Decades ago, our spacecrafts Pioneer-10 and Pioneer-11 informed alien civilizations that we live on the third arm of a tiny Solar System. We also told them that there are more than a hundred billion Solar Systems like ours in the Milky Way Galaxy alone and there are more than four hundred billion Galaxies in the visible part of the Universe. We live so far apart, there is no physical dangers from each other. By converting the Genetic Code with the Digital code, we will be happy to share the sequence of Human Genome with them. We can also share with them the genomes of all plants and life forms on Earth. They can see how we look like. If they wish, they can recreate us and our intelligence on their planet. Not only we share with them how we look like, but we will also be happy to share the genome sequences of all three million known and thirty million unknown living creatures on our home Planet Earth.

Conclusion

We may not be able to leave Planet Earth in the first few generations nor in the next hundred generations nor in the next thousand generations, but by 1,001 generation; we must prepare to leave. By then, as space travelers, we would have learned to recycle our food, our water, and our air in our city size spacecrafts linked to our Super Gigantic International Space Station. We would be self-sufficient and become independent to travel into the deep space in search of a new home. A home where our next thousands generation could live without fear of environmental collapse. Leave Earth, we must. If we don't destroy ourselves by going to nuclear war, we have enough time and enough energy in the Sun, for another four and a half billion years. That is all the time, we have. We race against time. Our Sun is rushing to become a Super Nova to explode and to destroy itself. It has been burning 70 million tons of Hydrogen gas every second for the past four and a half billion years. It has used up more than half of its total energy. Our thousand fleets of Spaceships must leave in thousand different directions. Kepler Space Telescope has identified more than 4,000 exoplanets, habitable planets within a life-time travel distance. We need outstanding visionary leaders to accomplish our missions. In the past, we had such leaders.

For example, when Britain was threatened by the Nazis during WWII, Sir Winston Churchill said, we shall fight in the air; we shall fight in the water and we shall fight on the land, but we will never surrender. We need that kind of leadership and that kind of determination. The entire humanity is threatened by even a greater danger, a total inhalation of the entire human race forever. We also say that we refuse to surrender. Sir Winston also said that if we fail; we all will fail and if we die; we all will die together. We say that we all refuse to die when the Earth dies.

In search of second Earth, we can explore our nearby star systems, alpha century is located four and a half light year from Earth, for a suitable planet for humanity. Within ten light years distance from Earth, more than a thousand exoplanets suitable for human habitations have been discovered. We may not get there as a people in a few generations. We can certainly try to plant seeds which will colonize distant Star systems.

We must first focus on developing Robotics and nanotechnology, the new generation of scientists who will create (micro size spacecraft for the microbial astronauts) robotic machines of molecular size which will be sent first to search for habitable planets. These nano machines will study and send the environmental conditions of the new Earth like planet to Mother ship so that we design a new generation of spaceship and prepare new generation of astronauts for the expedition who will explore if humans will be able to survive under different environmental conditions on a new planet.

Humans are inquisitive creatures. They have adventurous nature and pioneering spirit. They have this incredible urge to study unknown, whether it is underground, or under water or in the outer space. We all would like to travel into space. Some of us would like to take vacation in space other would like to mine precious metal in nearby asteroids. Space is the ultimate frontier, and it offers ultimate challenges where enormous opportunities await us. We could accomplish the unthinkable goals. We will learn to prolong human life. We will learn to make durable spacecrafts that will last a long time and we will also learn to create new matters like graphene and find new sources of energy. Using new material yet unknown, we will develop spacecrafts for light years journey into deep space.

Future Residents of Earth

What would happen to those people who stay behind. By 2050, the population of the world will reach nine billion. By that time, we would have developed new food, new fuel, and new medicine to treat every disease known to mankind to protect, preserve and prolong human life beyond one hundred years. This section discusses the impact of prolonging human life beyond one hundred years. The most nutritious vegetarian food containing all nine essential amino acids, will make world population lean healthy and long-lived. Besides new food, we would have new generations of plants which will serve as factories for producing new medicine called the genomic medicine which will treat specific disease based on the specific individual genetic make-up. This class of medicines will further increase human lifespan. Vegetarians live much longer than the non-vegetarians. We face the same population problem when we succeed in shutting off genes of all three old age diseases that is Cancer, Cardiovascular disease, and Alzheimer. Most people on genetically engineered food will live longer and happier life. It raises several questions. What happens to those people who decided to stay behind on Earth?

After we achieve that goal of reaching human lifespan to 100 years? What would be the quality of our life? By exercises and good nutrition, if the body mass is not retained, the Centenarians are most likely to be fragile and weak. They need the help of caretakers to perform the daily routine. By 2050, if we increase the age of about a hundred year of about a billion people, we need another billion caretakers. Will the society be happy with this achievement? I doubt it. The society is hardly likely to accept such a proposal. We must colonize exoplanets.

As I said above, for deep space travel in search of habitable planets, we need to increase human lifespan beyond one hundred years. To cure diseases to prolong human life, several present and future attempts are described below:

We need to make two rationale approaches: First, to identify rare allele in the Genome of Centenarians responsible for prolonging their lives. Once identified the allele, we need to conduct genetic engineering that is to cut, paste, copy, and splice the allele into the Genome of volunteers to study its function. Second, to design drugs to shut off genes of old age diseases to prolong life.

Next attempt to increase human life would be to prevent the loss of Telomeres, the six-letter code (TTAGGG) found at the tail end of each chromosome that shorten our DNA at each replication and shorten our lifespan. During replication, each Chromosome loses about 30 Telomeres each year. If we prevent the loss of Telomeres by using the enzyme Telomerase Reverse Transcriptase (TRT), we

could slow down the aging process. We have already demonstrated in the worm C. Elegance that we could increase its lifespan several fold. Now, we could translate this work in human being; we could try by making a less virulent Flu Virus carrying TRT gene when injected to a volunteer who comes down with a mild Flu. When he recovers from the Flu, the TRT gene would have inserted in the entire genome of every cell in his body (we can confirm the insertion by sequencing). Suppose at each replication only 15 Telomeres are deleted instead of 30 Telomeres. This person is likely to live twice as long. Also suppose the sequencing of his genome would confirm that every cell of his body carries the TRT gene. Since the longevity treatment with the Flu virus is safe, inexpensive and would be easily available to everyone, should we provide the treatment to every man, woman, and child on the face of the Earth?

Such studies are likely to raise two serious ethical questions. First, we must ask ourselves, do people have a right to live and second do we have a right to live as long as we wish, no matter how old, how weak or how sick we are? The answer to first question is, according to the UN charter, we all have the right to life, liberty, and pursuit of happiness. It is the second question which is troublesome. Do people have a right to extent their lives as long as they wish? Most people are reluctant to answer this question either no or yes. Both answers have some support.

Those who said No, have a good reason. First, they argue that there are seven and a half billion people live on planet Earth and we are adding 90 million additional people each year. According to UN estimate, by 2050, the population of the world is likely to reach nine billion. Does our planet Earth have all resources to support such a population explosion? Can we provide food, fuel, and medicine to all the people of the world? In poor countries millions of people are starving now. By extending lifespan, we will have serious problems such as lawlessness, riots, and chaos in the streets. Moreover, the current population of Earth have polluted the water, polluted the air, and polluted the land. Today, they wonder if the water they drink is safe, the food they eat is safe and the air they breathe is safe. If we continue to pollute the Planet with the current rate which is 110 million ton of pollutant that we release in the atmosphere each year; how much pollutants, we would accumulate in the atmosphere in ten years or in hundred years.

On the other hand, those who say Yes; we should extend human lifespan have good reasons as well. Our Sun is dying and without sunlight we cannot survive. We have no Plan B to save human life on some other Planet. We look up to Heaven to find another home for humanity. To search for a suitable Planet for human life to survive, we need to train an army of Astronauts to travel into deep space with extended life span. They may have to travel for centuries to find a habitable planet for humans. We do not want them to die on their way to find a new home for humanity. We must continue to search for treatment to prolong human life. Some simple-minded people, mostly religious people would say God created this wonderful Earth for us. Why can't we live on Earth forever? God has created us, and He will protect us. Science say that we cannot stay on Earth forever. We have limited time on Earth.

If we want to survive, we must leave Earth. For deep space travel, we need to increase our life span beyond one hundred years despite the population explosion and environmental pollution, we must continue to work to extend human life beyond 100 years. The Universe is vast. Universe must be teeming with life, but the distances are so vast, it is impossible to travel between star systems within the same galaxy. We have not achieved interstellar travels. Traveling between galaxies is unthinkable currently. There must be star systems which are formed earlier than our Solar System must have planets whose population must be technologically more advance than us. We must find a suitable home for humanity in another Solar System. We need time to develop technology for interstellar travel.

Mother Nature has not been very kind to us. She should have created us at least a billion years ago. We could have populated many Solar Systems in the Milky Way Galaxy. The great tragedy is that we came out of Africa recently when the Sun has used up half of its energy. The first half Chimp/half human, Lucy, the mother of us all, walked out of Haggar Valley, Ethiopia, about three and a half million years ago. Despite this delay, we still have enough energy in the Sun to get out of this Solar System only if we don't destroy ourselves by either going to Nuclear War or inviting Environmental collapse, Meteorite impact followed by global forest fire or Tectonic plate shift resulting colossal Tsunami drowning life under ocean. We made more scientific discoveries during the last twenty-five years than the entire history of humanity on Earth. We have enough technology to take humanity out this solar system. We plan to take the following baby steps:

For those inhabitants of Planet Earth who chose to stay, the non-vegetarians. They could do us a favor; they could stay behind to broadcast what they witness the final days on Earth the final sunset, the end of life on Earth; the inhabitants will feel the intense heat, witness the worldwide burning of Oxygen, the boiling off the oceans, the massive forest fire. They could broadcast live the end of the Earth for the space travelers who might be light years away from Earth on the way to Alpha Century Star system in search of habitable planets for humans. As our Sun used up most of its energy, it will expand no further. They could broadcast us how the Super Nova will collapse on itself, and the Sun will explode with Titanic force. The gravity of the planets orbiting around Sun will collapse and all other planets and their moons will fall on each other causing further explosions destroying the entire Solar System. The inhabitants of distant galaxy such as Andromeda Galaxy will see the destruction of Earth as a tiny ripple on the third arm of the Milky Way Galaxy.

As the Chinese proverb says, a thousand-mile journey begins with a single step. The space X chief Elon Musk announced on the Mission to Mars. In 2024, we plan to send men on Mars. It is the first step in the right direction. It will technologically prepare us to survive on Mars under extremely cold condition on a tree-less water-less planet without Oxygen. Once we learn to survive on Mars, we could use Mars as a base to launch un-manned spacecrafts to distant Star systems in search of habitable planets for humanity. For deep space travel, Vegetarianism will be the order of day. Carrying livestock on a long-distance space journey is unthinkable. The food we consume will be developed based upon the genetic make-up of a specific individual; we must eat special Genomic food to keep us healthy and to prolong our life span for deep space travel. Musk is certain to colonize Mars by 2026 and within a decade, we will establish Mars as a self-sustaining civilization. Our future presents limitless possibilities. We, as human Martians, will take the first giant step for our thousand light years journey into deep space. We will re-define the limits of our knowledge and we will redefine the limits of our technology. We will pursue humanity's ultimate dream with limitless zeal, limitless enthusiasm and we are sure that eventually human spirit will triumph. Although the Earth was formed 4.5 billion years ago, we walked out of Africa only three and a half million years ago.

Within that brief time, we became technologically so advanced that we are about to colonize Mars. If we could survive for a million years without going to nuclear war destroying our planet, we would be technologically so advanced that we should be able to achieve our goal to sail through our solar system colonizing planets, colonizing comets, and colonizing asteroids for human habitation. Our journey will continue across solar systems, across galaxies and beyond into the cold dark space as far as our imagination will go into deep space to spread human intelligence in every corner of the Universe. Our aim is to protect, preserve and spread human intelligence in every corner of the Universe. For the first time we learn that our destiny lies in our own hands.

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About the author

Hameed Khan was born in India, educated in England. After receiving doctorate degree in Chemistry from the University of London, he was honored with the Institute of the Cancer Research postdoctoral award of the Royal Cancer Hospital, University of London. America honored him with the Fogarty International postdoctoral award to work at the National Institutes of Health (NIH), and the National Cancer Institute of USA. He is a discoverer of AZQ (US Patent 4,146,622 & 4,233,215) for treating Brain Cancer for which he was honored with the "2004 NIH Scientific Achievement Award." NIH has been his home for over quarter of a century. He was also honored with a Gold Medal by the Government of India. He is a Fellow of the American Institute of Chemistry and was elected to the American Science Advisory Board. He works at NIH. The ideas expressed in this chapter are his own and do not represent government policies.

Chapter 2

Endothelial Dysfunction in Children and Young Adul ts: Clinical Implications and New Perspectives

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ABSTRACT

Evaluation of endothelial dysfunction (ED) as a systemic disease has gained increasing attention in view of its emerging relevance for cardiovascular control. ED as an important early sign of generalized vascular disease contributes to most forms of atherosclerotic cardiovascular disease (CVD) including hypertension and chronic heart failure. ED reflects a vascular phenotype prone to the initiation and progression of atherogenesis and its complications. Atherosclerosis begins in childhood and ED as the precursor and earliest clinically detectable form of atherosclerosis can be demonstrated already in early childhood, through a long preclinical stage.

We discuss future considerations, such as unifying mechanisms in numerous pathophysiologic processes that participate in ED with major clinical implications. We herein summarize current understanding of how to assess ED, main risk factors, mechanisms underlying the development of ED, prognostic implications of ED, biomarkers that reflect ED, which have been proposed to indicate ED and/or vascular damage, respectively. The current normative data on the possibilities of early non-invasive detection of ED with a focus on the currently widely used method of plethysmography with its strengths and limitations are mentioned. A new option for a non-invasive determination of ED is a combined diagnostic approach by measuring the plethysmographic flow-induced Reactive Hyperemia Index (RHI) using EndPAT index as the ratio of endothelium-dependent postocclusive and preocclusive vascular tone changes. In this regard, circulating ED markers include asymmetric dimethylarginine (ADMA)-related biomarkers, high-sensitive CRP (hsCRP), vascular adhesion molecule-1 (VCAM-1) and E-selectin.

Significantly decreased RHI with elevated plasma concentrations of biomarkers imply a possible association with premature ED in young patients and confirm the progression of atherosclerosis into adulthood.

Microvascular ED has been associated in young patients not only with metabolic syndrome, obesity, hypertension, diabetic vascular complications, dyslipidemia or atherosclerosis but also with many different disease processes and risk factors including chronic inflammatory, autoimmune and widespread systemic diseases, infection, sepsis, hemolytic-uremic syndrome, rheumatic diseases, celiac disease, Henoch-Schonlein purpura, Kawasaki disease, low birth weight, sleep-disordered breathing, leukaemia, beta-thalassemia major, idiopathic pulmonary hypertension, and so on, without overt CVD. Especially in pediatric cystic fibrosis (CF) patients, our group demonstrated the progressive development of microvascular ED from childhood to adulthood. Better understanding of a close relationship between the underlying diseases, ED and CVD, early identification, the potential discovery of therapeutic targets and the restoring of ED could serve as a preventive approach to protect against the occurrence of ED and the subsequent complications such as atherosclerosis in "at risk" paediatric population. The combined approach seems to improve risk prediction for the earliest assessment of ED before classically recognized markers of disease, and may be useful for the further clarification of premature atherosclerosis and future CVD.

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Keywords: Endothelial Dysfunction, Atherosclerosis, Cardiovascular Diseases, Reactive Hyperemia Index, Biomarkers, Children

Introduction

Endothelial dysfunction (ED) is an early functional preclinical manifestation of atherosclerosis (AS), a well-established systemic disorder that represents an early marker of the development of cardiovascular disease (CVD). CVDs due to atherosclerosis, especially ischemic stroke and coronary heart disease are among the leading causes of premature death. The most effective approach to reduce CVD mortality and morbidity is preventive measure aimed at active searching for at risk asymptomatic individuals. Thus, in the earliest stages, there is a merely functional fully reversible damage of the endothelial lining due to mechanical, physicochemical and immunological factors. Most commonly, the impaired endothelium-dependent vasodilatation due to a reduced nitric oxide (NO) production is defined as a hallmark of ED. As Hamburg et al., demonstrates, ED is characterized by a reduced vasodilatory response to a suitable ischemic stimulus, as added by Incalza et al., an imbalance between reactive oxygen species (ROS) and antioxidants, as well as a procoagulant and proinflammatory state becoming a proatherosclerotic structure [1,2]. ED reversibility seems to be a primary target in the effort to optimize therapeutic strategies and to reduce CVD risk. Early detection of ED may have therapeutic and prognostic implications. Therefore, early detection of pathological conditions in the early reversible phase without the developed manifest form of atherosclerosis makes it possible to delay or even prevent the manifestation of AS. Morphological atherosclerotic changes can already be demonstrated in children, when clinical manifestations are still quite minimal, but unfortunately ED in childhood has so far received a little attention from researchers [3].

Pathophysiology

ED is thought to be a key event in the pathogenesis of vascular diseases and it precedes clinically obvious cardiovascular problems such as atherosclerosis, systemic and pulmonary hypertension, cardiomyopathies, vasculitis and heart failure [1]. Abnormalities in the structure and function of endothelial cells (ECs) also play an important role in the ethiopathogenesis of uncontrolled chronic inflammation. Dysfunctional endothelium

leads to increased vascular wall permeability with a tendency to vasospasm, thrombosis and insufficient inhibition of subendothelial proliferation, to increased production of proinflammatory cytokines, vasoadhesive and prothrombogenic molecules [4].

The endothelium, once considered a mere selectively permeable barrier between the bloodstream and the outer vascular wall, is now recognized to be a crucial homeostatic organ, fundamental for the regulation of the vascular tone and structure. Vascular endothelium is known as the largest and most metabolically active tissue, a selective barrier, a thin membrane presenting a thromboresistant surface, lining on the luminal surface of blood vessels and heart, presenting many biologically active substances for the regulation of vascular relaxation and contraction, thrombogenesis and fibrinolysis, activation and inhibition of blood cell elements. Healthy endothelium is essential for cardiovascular hemostasis as well as maintaining the vascular integrity by controlling platelet aggregation, vascular tone, blood fluidity and fibrinolysis, adhesion and transmigration of inflammatory cells, and angiogenesis. A balance between the production of chemotactic, pro- and antiplatelet and pro- and antiproliferative substances is essential to maintain blood flow, vascular patency and blood pressure [5]. ECs express vasodilators (NO, prostacyclin) and vasoconstrictors, such as thromboxane A2 (TxA2), prostaglandin H2 (PGH2), endothelins, superoxide anion, angiotensin II, further proliferation-stimulating cytokines, mainly platelet-derived growth factor (PDGF, a major growth factor released by platelets at the sites of vascular insults), two key enzymes of fibrinolysis (tissue-type plasminogen activator and inhibitor). Also, vasoactive substances (e.g., bradykinin) are degraded in the endothelium.

The endothelium is an essential component of the blood coagulation system. The healthy ECs regulate the interplay of the complex coagulation system. Due to CVD risk factor, normal endothelium initiates mechanisms to clot by expression of tissue factor (TF) and fVIII and anticoagulate by expression mainly of thrombomodulin. At the same time, activated ECs by stimuli recruit platelets to the injury places, by expression of von Willebrand factor (VWF), and act surface for an aggregation and pro-coagulant status.

Thus, ED is responsible for the blood coagulation. ED leads to the activation of endothelial cells, which contribute to the pathogenesis of thrombosis. There is evidence that pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL)-1, up-regulate the production of TF and VWF, while attenuating the expression of thrombomodulin, NO and prostaglandin I2 [6].

Patients with systemic inflammation show an impaired protein C synthesis as well as its activation. While protein C is synthesized by hepatocytes, ECs can regulate protein C activation through the expression of thrombomodulin. Thrombomodulin levels are significantly down-regulated by the presence of pro-inflammatory cytokines, such as TNF-α and IL-1, resulting in decreasing of protein C activation and subsequently to a shift to pro-thrombotic states. Protein C has also been implicated in the development of disseminated intravascular coagulation with associated organ dysfunction [7].

Inflammation has also been associated with ED and can be accompanied by thrombosis. Endothelium directs the accumulation of leukocytes in inflammation by the expression of specific cell surface molecules which are adhesive for ligands on circulating leukocytes.

Examples of such molecules are E-selectin and intercellular adhesion molecule1 (ICAM-1). Endothelium expresses E-selectin in inflammation. Among other endothelial adhesion receptors belongs also vascular cell adhesion molecule 1 (VCAM-1) [8]. Thus, inflammation and endothelial function play significant roles in the pathogenesis of AS. Elevations in certain inflammatory mediators as well as evidence of ED are related to increased risk of future cardiovascular morbidity.

ED is defined as a reduced capacity for NO production and decreased NO sensitivity [9]. NO is formed from the amino acid L-arginine in ECs by calcium-calmodulin-dependent enzyme endothelial-derived NO-synthase (eNOS) in reaction to mechanical, biochemical and pharmacological stimuli.

Summarizing, the reducing bioavailability of NO as a hallmark of ED may be caused by reduced expression of eNOS, impairment of eNOS activation, or inactivation of NO by oxidative stress. NO is known as the smallest signaling molecule with strong vasodilatory effect. NO released from ECs works with prostacyclin as an inhibitor of platelet aggregation, leukocyte adhesion to the vascular wall and vascular inflammation by the inhibition of the attachment of neutrophils to ECs and the expression of adhesion molecules. Leukocyte adherence is an early phase of atherogenesis, therefore NO is a key regulator of vascular homeostasis. Anti-adhesive effect is due to either interference with the ability of the leukocyte adhesion molecule CD11/CD18 to bind to the endothelial cell surface or suppression of CD11/CD18 expression on leukocytes. NO has antiproliferative effects mediated by cyclic guanosine monophosphate (cGMP). NO inhibits DNA synthesis, mitogenesis, and proliferation of vascular smooth muscle cells. The inhibition of platelet aggregation and adhesion protects smooth muscle from exposure to platelet-derived growth factors. Therefore, NO also prevents the later step in atherogenesis, the formation of fibrous plaques. Based on current studies, NO has anti-atherosclerotic effect, as well as pro-angiogenic and pro-neovascularizing effects.

In recent years, the role of endothelial glycocalyx (EG) has been discussed. EG is a gel-like layer on the luminal surface of ECs which is composed of glycoproteins, proteoglycans (mainly syndecans and glypicans), glycosaminoglycans and long chains of hyaluronan. Endothelial functions have been shown to be modulated and mediated by EG. EG damage is involved in pathophysiological conditions like sepsis, chronic and metabolic diseases. EG is essential for maintaining homeostasis and it plays a role of vascular barrier, mainly a key role of a regulator of coagulation, inflammation, microvascular permeability and intracellular cell signaling [10]. EG has a crucial role in the development of organ fibrosis, as damage of EG is associated with ED, leading to decreased NO bioavailability, excessive ROS production, inflammatory cytokine release, platelet adherence, coagulation, and leukocyte adhesion. In patients with AS, damage of the vascular EG is a common sign alongside ED. EG dysfunction causes microvascular leakage which can lead to interstitial pulmonary abnormality with a ground glass opacity [10]. Masola et al., described the mechanisms that disturb EG stability such as ROS, matrix metalloproteinases, and heparinase [11]. Vascular damage could be caused by glycocalyx degradation, as well as by impairing fibroblast growth factor receptor 1 (FGFR1)/exostosin1(EXT1)-mediated glycocalyx reconstitution, while FGFR1 is known as a mediator of endothelial repair [12]. A high syndecan-1 level, as a marker of EG degradation, is associated with inflammation, protein C depletion, and fibrinolysis [13]. Levels of syndecan-1 may correlate with E-selectin.

Patients with cardiovascular risk factors as well as patients with vascular disease show signs of ED in the sense of a reduced ability of the endothelium to produce an adequate amount of bioactive NO capable of mediating vasodilation. Cardiovascular risk factors and vascular disease are also associated with an increased production of ROS. ROS reduce NO bioavailability and cause ED. There are several enzymes that can produce ROS in the vessel wall. These include mainly nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidase, and mitochondrial respiratory chain enzymes [9]. Reduced NO production under cardiovascular risk conditions leads to serious endothelial problems, so many studies have been performed to evaluate the possibility of reversing ED by improving endothelial NO release.

Over the last decades, among vasoactive factors released by the endothelium, the roles of hydrogen sulfide (H_2S) in the pathogenesis of ED have been studied in recent years, as one of the main factors regulating homeostasis. As a result, the current understanding of H_2S-mediated endothelial cell function in both health and disease has grown exponentially. Citi et al., demonstrated the role of H_2S in CVD pathophysiology and showed the possible therapeutic approaches using molecules able to release H_2S. H_2S plays an important role in the regulation of vascular homeostasis as an endogenous gaseous molecule, synthesized from L-cysteine by two enzymes: cystathionineb-synthase and cystathioninec-lyase. The impairment of H_2S endogenous production is related to the pathogenesis of ED, as Wang et al., demonstrated. Many studies have established the vasculoprotective effects of H_2S gasotransmitter. Lv et al., declares the relationship between the impaired H_2S production and vascular remodeling [14-15].

To summarize, H_2S inhibits atherogenic modification of low-density lipoproteins (LDL) , prevents monocytes adhesion due to endothelial activation, promotes vasorelaxing responses, decreases intimal hyperplasia by inhibiting vascular smooth muscle cells migration and proliferation, limits vascular calcification and osteoblastic differentiation of vascular smooth muscle cells, thrombogenesis, platelet aggregation and inflammation [15-20]. H_2S has also an antiapoptotic effect. Furthermore, H_2S potentiates the NO-mediated vasorelaxing effect through the inhibition of phosphodiesterase-5 (PDE-5), prolonging the half-life of cGMP which is the key factor in NO pathways. H_2S compensates for the lack of NO-endothelium production and causes inactivation of nuclear factor kappa-B (NF-κB) leading to decreased expression of the inflammatory factor ICAM-1 [21]. Key mechanisms for the H_2S antiatherosclerotic effect include modulations of vascular smooth muscle cells apoptosis, interactions with NO, ion channels regulation, reduction of oxidative stress and inflammation, to protect the vascular endothelium. H_2S attenuates atherosclerotic lesions by blocking oxidative modification of low-density lipoprotein (LDL) and elevating antioxidative ability [22].

In fact, H_2S is mainly produced in endothelium and is involved in the fine regulation of endothelial integrity and functions. For this reason, altered H_2S bioavailability has been proposed as a novel marker of ED advancement and prognosis [15]. Recent data provide evidence that H_2S can prevent CVD. Thus, regulation of H_2S level provides a novel therapeutic method against ED-related diseases. The role of H_2S in endothelial homeostasis and its putative therapeutic applications was reviewed by Altaany et al., [23]. Therefore, the application of H_2S-releasing drugs or using gene therapy to increase endogenous H_2S level may help restore endothelial function and antagonize the CVD progression.

Since the Rho-associated coiled-coil kinase (ROCK) discovery in 1996, ROCKs have been extensively studied in relation to ED. There are many signaling molecules involved in the pathogenesis of ED via impairment of NO bioavailability. Some of them, like phosphoinositide 3-kinase (PI3K)/Akt (protein kinase B), ROS and arginase, are connected with the RhoA/ROCK pathway. Lysophosphatidic acid (LPA) as a proinflammatory lipid mediator can activate ROCK pathways in endothelium. ROCK is a serine/threonine kinase with two isoforms, expressing in vascular endothelial and smooth muscle cells. Normal ROCK activation is necessary for the regulation of endothelial barrier integrity and RhoA is a key element for T cell transendothelial migration [24]. Yao et al., finally clarified the important pathophysiological role of the ROCK in vascular ED [25]. RhoA/ ROCK activation by C-reactive protein enhance endothelial plasminogen activator inhibitor-1 expression, which may result in atherothrombogenesis [26]. As Yao demonstrated, the RhoA/ROCK pathway plays an important role in impaired NO production, it regulates the expression and activity of eNOS. This occurs by reducing PI3K/Akt activation and subsequent reduction of eNOS phosphorylation and downregulation of eNOS mRNA stability [27]. Additionally, activation of the RhoA/ROCK pathway causes elevation of arginase activity, which results in limited availability of the substrate L-arginine for eNOS function [25]. Some factors such as ROS, angiotensin II, thrombin, TNF α, lysophosphatidic acid cause an imbalance between endothelium-derived relaxing factors and endothelium-derived constricting factors, with impaired endothelial function and enhanced smooth muscle cells contractility which can progress to hypertension and other vascular diseases. On the other side, ROCK inhibitor can reduce vasoconstriction caused by acetylcholine in vessels with ED. Inhibition of ROCK can prevent thrombin-induced ICAM-1 expression and can inhibit NF-κB activity and tissue factor expression in ECs, which confirms the RhoA/ROCK pathway affects thrombus formation [28-29]. Inhibition of the RhoA/ROCK pathway may have significant clinical implications in ED prevention.

Oxidative stress leads to a reduction in NO bioavailability and/or signaling with a subsequent pattern of ED. Schulz et al., summarizes the mechanisms of vascular changes due to oxidative stress which include increased vascular cell proliferation and migration, apoptosis, inflammation and extracellular matrix alterations, causing vascular hypertrophy and remodeling [30].

Atherogenesis

Atherogenesis is a complex process of chronic inflammatory and reparative responses affecting the vessel wall. Atherosclerosis is a chronic, progressive, pathological process of large and medium-sized arteries with a dominant inflammatory component, which manifests itself mainly in coronary, carotid and peripheral arteries [31].

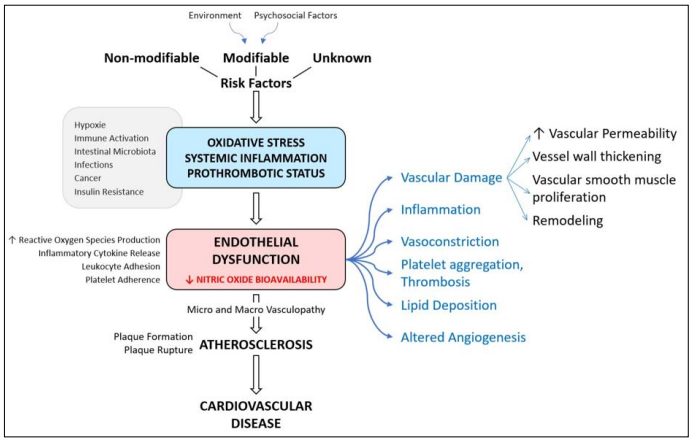

Figure 1: Atherogenesis – the process from risk factors to endothelial dysfunction, progressing to atherosclerosis and cardiovascular disease.

As reported early in several studies including Wang et al., and Gao et al, H_2S deficiency is related to the pathogenesis of AS [32-33]. Gao et al., suggested that H_2S deficiency may predispose stable coronary artery disease patients to vulnerable plaque rupture. The initial phase of atherogenesis is endothelial activation characterized by abnormal pro-inflammatory and pro-thrombotic activity of ECs leading to reduced NO bioavailability, vascular tone damage and to ED (Figure 1). Inflammation and endothelial function play significant roles in the pathogenesis of AS. Elevations in certain inflammatory mediators as well as evidence of ED are related to increased risk of future cardiovascular morbidity presents mRNA as novel mediators of vascular damage [2, 34].

The role of endothelial function in AS has been elucidated by clinical studies that have demonstrated vascular endothelium may modify the effects of risk factors on the AS development. ED refers to the loss of physiological endothelial functions caused by different risk factors, including hyperglycemia, hypercholesterolemia and hyperhomo-cysteinemia as Cheng et al., demonstrated via systemic review [35]. As demonstrated by Schulz et al., systemic arterial hypertension is a dominant cardiovascular risk factor that causes significant morbidity and mortality, and is becoming an increasingly common health problem because of the increasing longevity and prevalence of risk factors such as sedentary lifestyle and obesity [30].

Risk factors in coronary heart disease were first defined by the Framingham heart study (FHS), published in 1957. FHS demonstrated the epidemiologic relations of cigarette smoking, blood pressure and cholesterol levels to the incidence of coronary artery disease [36-37]. The most serious general risk factor for atherogenesis is dyslipoproteinemia and the main risk factor for CVD death is obesity. There are many CVD risk factors that can be modified such as hypertension, high blood cholesterol levels, smoking, diabetes mellitus, obesity, lack of physical activity, unhealthy diet and stress. On the other side, conventional risk factors that cannot be controlled include age (the risk increases with age), sex (men are generally at higher CVD risk), genetic factors (familial hypercholesterolemia, familial dysbetalipoproteinemia and familial hyperlipidemia), family history of CVD premature manifestation. Ridker confirmed the possible proatherogenic effects of infection as a CVD risk factor, such as chronic infections of Chlamydia pneumoniae, Helicobacter pylori and Pophyromonas gingivalis, which can activate the immune system with subsequent immunopathological endothelial damage [38]. Classification of AS risk factors is shown in Figure 2.

Dyslipoproteinemia is a disorder of lipoprotein transport and metabolism, manifested by abnormal lipid levels. Conditions associated with high levels of total cholesterol, LDL cholesterol and low concentrations of high-density lipoprotein (HDL) cholesterol have a significant atherogenic character. Acquired dyslipoproteinemia results from another disease (mostly endocrinopathy, kidney and liver disease), poor lifestyle, especially inappropriate nutrition in combination with insufficient physical activity [39]. Risk factors in childhood are specified by the Bogalusa Heart Study [40]. Inflammatory processes mediated by inflammatory mediators play a crucial role in the pathology of AS [41-42]. Weber and Noels, connect the inflammatory process with the lipid metabolism disorder in the pathogenesis of AS [43].

Morrison et al.,proves that age is an important predictor of carotid intima-media thickness (IMT) in youth. Among traditional CV risk factors, dyslipoproteinemia and family history of premature coronary heart disease are independent predictors of IMT [3]. IMT, a surrogate marker of subclinical atherosclerosis in adults, is increased in youth heterozygous for familial hypercholesterolemia and declines with lipid lowering pharmacotherapy.

The increased risk of CVD is associated with systemic inflammation, prothrombotic status, oxidative stress and ED. Chronic inflammatory and autoimmune diseases include risk factors regarding premature manifestation of CVD, in particular acute myocardial infarction, stroke and ischemic heart disease (IHD) [44].

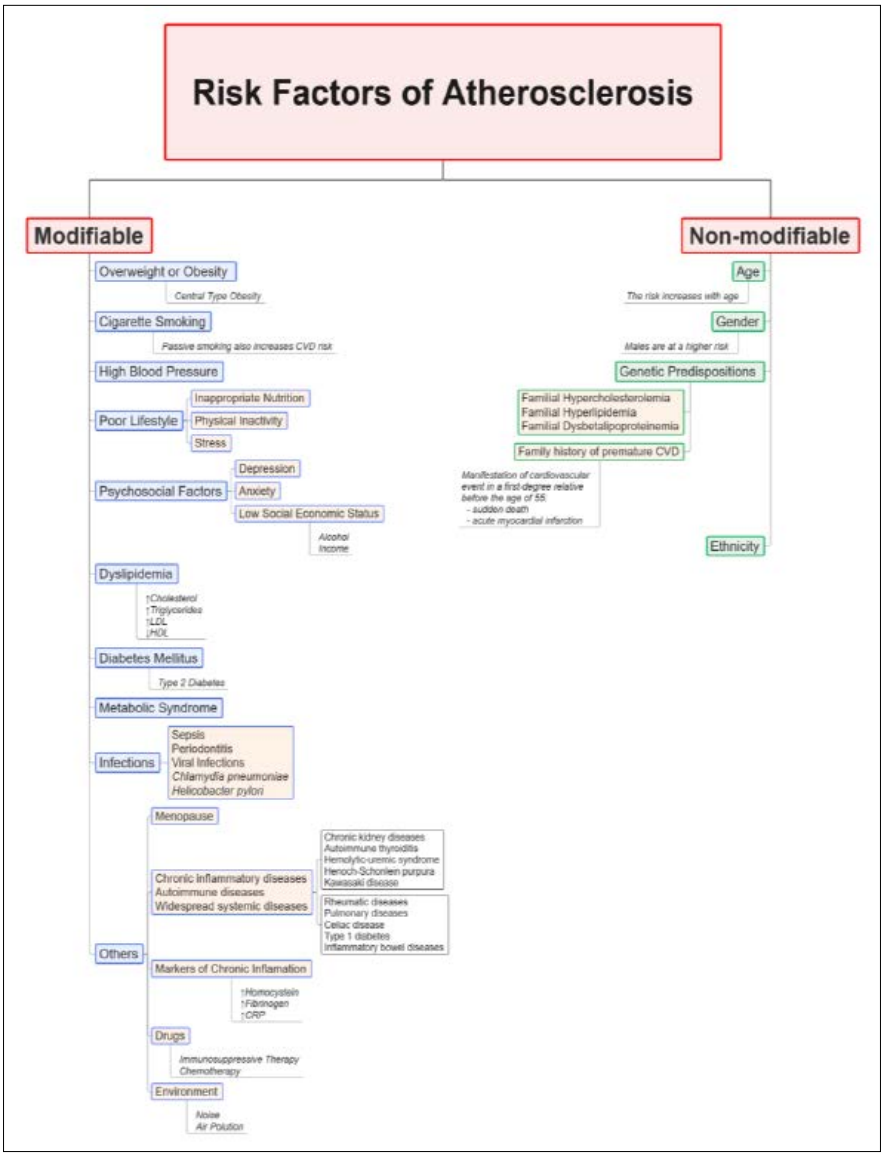

Figure 2: Classification of Risk Factors of Atherosclerosis

Risk diseases for the premature AS manifestation include chronic inflammatory and autoimmune diseases associated with activation of systemic inflammatory biomarkers, especially familial hypercholeste-rolemia, diabetes mellitus, obesity, rheumatic diseases (systemic lupus erythematosus, rheumatoid arthritis, progressive systemic sclerosis, psoriasis), pulmonary diseases (cystic fibrosis, asthma, chronic obstructive pulmonary disease, alpha1-antitrypsin deficiency, obstructive sleep apnea), chronic kidney failure, inflammatory bowel disease (IBD) and others (periodontitis, depression, viral infection and sepsis). Despite intensive investigation of the systemic inflammatory process with the activation of systemic inflammatory markers, little attention has been paid so far to the occurrence of CVD in childhood.

Methods of Detection of Endothelial Dysfunction

Due to ED reversibility, the ED phase is an important period for therapeutic intervention. ED is clinically asymptomatic at an early stage, but it can already be detected. Evidence for the existence of ED in children and young adults has led to the development of diagnostic methods in an effort to determine individual cardiovascular risk. Currently, there are a number of imaging and laboratory methods that evaluate vascular properties at the level of the endothelium or vessel wall. Most of these methods are not used in routine clinical practice due to their complexity, mainly difficult interpretations of findings and high biological variability in childhood, technical, personnel and economic demands and low biomarkers specificity. ED detection is currently performed in at risk populations (smokers, diabetics, patients with dyslipoproteinemia, etc.). ED can be detected directly using invasive and non-invasive imaging methods and/or evaluated indirectly using selected biochemical markers.

Imaging Methods

Invasive and non-invasive imaging methods can be used to detect ED. ED was previously measured by arterial catheterization to identify the response of the artery to acetylcholine. Invasive methods include angiography, quantitative coronarography, intravascular ultrasound, and methods derived from virtual histology and palpography [45]. As these are spatially, personnel and instrumentally demanding examinations, non-invasive methods represent a more advantageous alternative for ED detection. They are based on the assumption that the measured values from the examined peripheral vessels (most often the brachial and radial artery) are identical with the values of other less

accessible vessels, as ED is a systemic event. It was demonstrated that endothelial function in the coronaries is closely related to endothelial function in peripheral arteries such as the brachial artery [46]. The technique of intracoronary infusions of vasoactive substances can therefore be used in the brachial artery, which is easier accessible and with fewer complications. Changes in forearm blood flow during acetylcholine or nitroprusside infusions are an index of vasomotor function.

Non-invasive possibilities of ED detection include morphological methods (IMT, optical coherence tomography) and methods monitoring the functional manifestations of blood vessels (Doppler sonographic examination). Another currently preferred method is plethysmography. In the 1990s, high-frequency ultrasonographic imaging of the brachial artery to assess endothelium-dependent flow-mediated vasodilation (FMD) was developed, including the introduction of guidelines. This non-invasive technique of the assessment of endothelial function stimulates the release of NO, resulting in post-occlusive vasodilation that can be imaged and quantitated as an index of vasomotor function. This method is based on the physiology of blood flow regulation in vessels: peripheral arteries respond to physical and chemical stimuli by adjusting vascular tone and regulating blood flow [47]. Increased blood flow in peripheral arteries leads to an increased NO production and subsequent vasodilatation. The vasodilatory response of vessel to increased shear stress is called FMD or endothelium-dependent vasodilatation, since it is based on the ability of NO production by endothelium. The advantage of this method is its non-invasiveness with minimal burden on the patient, efficiency and easy using. Limitations are large biological and technical variability of the measurement. Ultrasonographic evaluation of postocclusive dilatation of the brachial artery was first described by Celermajer et al., who demonstrated a FMD eduction in smokers and children with familial hypercholesterolemia. FMD correlates with the image of coronary AS and the occurrence of cardiovascular attacks [48-49].

IMT is a sonographic examination of the carotid arteries by evaluating the thickness of the intima and media. FMD and IMT are sonographic non-invasive methods, but technically and time-consuming which limits their clinical use. Another non-invasive method used for evaluating endothelial function in brachial artery is Gauge-Strain Plethysmography, firstly developed by Hokanson in 1975 [50]. Electrically calibrated plethysmography uses impedance changes to evaluate the forearm blood flow during reactive hyperemia. The benefits of this technique are non-invasivity, simple usage and easy reproducibility, its results are less observer-dependent than ultrasound.

Another non-invasive method for evaluating microcirculation is Sidestream dark-field (SDF), an optical technique to visualize microcirculation with higher contrast as well as quality of sublingually-acquired microcirculation images and clearly observable venular granularity compare to orthogonal polarization spectral (OPS) imaging [51]. From the SDF images of vessels, certain physical quantities such as vessel length, vessel diameter, and velocity of red blood cells can be estimated [52]. In comparison with other imaging techniques of microcirculation such as photoacoustic imaging and optical coherence tomography, SDF imaging is simple, low cost and easily applicable.

Insufficient oxygen supplies in microcirculation may lead to tissue hypoxia which is considered to be one of the factors of serious diseases, such as CF and chronic bronchitis [48]. Visualizing an individual microcirculation and observing the changes in the oxygen saturation (SO2) during disease progression can be useful for understanding disease dynamics. Hashimoto et al., confirmed that SDF oximetry can measure SO2 changes that occur in accordance with alteration of the microcirculation [53].

In recent years, the evaluation of ED has come to the forefront of research with a new non-invasive method of measuring Reactive Hyperemia Index (RHI) based on the plethysmographic principle of peripheral arterial tone (PAT) evaluation. Insufficient increase in PAT amplitude during the phase of reactive hyperemia is associated with ED.

Reactive Hyperemia Index Measurements

Non-invasive measurement of RHI is based on the principle of plethysmographic evaluation of the post-occlusive endothelium-dependent changes in peripheral arterial tone (PAT) using an EndoPAT2000 recorder (Itamar Caesarea®, Israel). The PAT signal is measured by biosensors placed on the index fingers of the hands by recording changes in the arterial pulse volume of the finger. A unique feature of PAT biosensors is a creation of a uniform sub-diastolic pressure field in the distal two-thirds of the fingers, including their tips. Applying pressure has benefits in preventing an accumulation of distal venous blood that can induce venoarteriolar vasoconstrictive reflex. Also, it releases the arterial wall tension which can generate a larger dynamic range of the measured PAT signal and it fixes biosensor to the finger, which reduces motion artifacts (Itamar Medical).

The measurement is performed on the subject in the supine position in a quiet, thermoneutral room. Initially, body mass index (BMI) and blood pressure on the non-dominant arm are measured. During the first rest phase, which lasts 15 minutes, the input PAT values on both upper limbs are recorded. Subsequently, a five-minute occlusion of the brachial artery is performed on a non-dominant limb using a pressure cuff inflated 60 mmHg above the systolic pressure examined, minimum 200 mmHg, maximum 300 mmHg, and the second five-minute occlusion phase takes place. After its completion, the cuff of the tonometer is blown out sharply and the third post-slip phase lasting 5 minutes continues. After the rapid release of the cuff pressure, reactive hyperemia and endothelium-induced dilation occur. This dilation is registered by the device as an increase of the PAT signal amplitude. RHI reflects changes in arterial tone in the peripheral bed. Insufficient increase in PAT amplitude during the third post-occlusive phase is associated with ED. The EndoPAT index (RHI) is calculated automatically from the ratio of the post-occlusive and pre-occlusive arterial flow (PAT signal) relative to the values of the simultaneously measured non-occluded contralateral arm. RHI is a mark of endothelial function, RHI values below the cut-off level are a sign of impaired endothelial function. The RHI cut-off is set to 1.67 in adults, at which value the sensitivity of the method is 82% and the specificity is 77%. According to literature data, the mean RHI in healthy adults is 2.06, according to Domsic et al., [54].

The strengths of EndPAT, a test of microvascular endothelial function, in addition to its non-invasiveness with clinical use in children and adolescents, is its high sensitivity, low biological variability and objectivity of results due to automatic data evaluation. Simultaneous measurement of the contralateral non-occluded non-dominant arm allows the elimination of endothelium-independent changes in vascular tone.

Limitations of the method are the absence of a precise cut-off limit for RHI in children and uniform sensors with no definition of the minimum finger thickness. To avoid inaccurate results caused by

measuring younger children with fingers too small for sensors, it is necessary to select participants from school age. Kelly et al., demonstrated that younger age is associated with lower RHI but not lower FMD among adolescents and results of this study suggest that age is associated with RHI [55]. Therefore, it is suggested to choose the control group with age comparable to high-risk patients [46]. Hamburg et al., demonstrated an increased baseline pulse amplitude (PAT) in obese persons with metabolic syndrome and an inverse relationship between baseline and response PAT to hyperemia [1].

Some studies have not found a correlation between FMD and RHI with respect to ED which may be explained by different properties of large conductive brachial arteries and the peripheral resistive artery bed. Both RHI and FMD measure post-occlusive changes in arterial tone.

Biomarkers

Increased plasma levels of biomarkers or products of endothelial overactivation are a sign of ED. In recent years, in connection with the effort to determine the risk of developing AS in high-risk groups of patients, the predictive potential of markers of endothelial activation has been intensively investigated. Invasively obtained blood samples directly during coronarography accurately detect the endothelial function, but in practice, plasma or serum concentrations of biochemical markers taken from venous blood are used more frequently. However, their disadvan-tage may be their biotransformation or instability.

ED is manifested by specific biochemical markers that can be used in the detection of proinflammatory and prothrombogenic changes in the endothelium [56]. Biochemical parameters of ED include vasoactive molecules (endothelin-1, prostanoids), coagulation parameters (VWF, tissue plasminogen activator and plasminogen activator inhibitor), adhesive molecules mediating mainly the attachment of leukocytes to the endothelium (VCAM, ICAM, E-selectin), NO evaluation (NO synthase activity, ADMA), inflammatory parameters (IL-6, hsCRP). Currently, independent biomarkers of cardiovascular risk are gaining prominence, increasing the predictive value of current AS risk factors. Biochemical parameters evaluated in relation to ED include hsCRP, ADMA, VCAM-1, and E-selectin. Levels of VCAM-1 and ICAM-1 were found to be negative predictors of acute coronary syndrome [57].

C-reactive protein determined by high sensitivity method (hsCRP) is important for the assessment of the inflammatory component of atherogenesis. It is a sign of pro-inflammatory endothelial activity, an overall inflammatory marker associated with the atherogenic process. In practice, it is important to eliminate the increase in hsCRP due to the ongoing acute inflammatory process unrelated to ED. Higher concentrations of hsCRP could predict CVD morbidity and mortality in clinically asymptomatic individuals, but due to its low specificity, hsCRP is not considered a key marker for ED detection [58].

ADMA is a competitive (non-selective) inhibitor of endothelial NO synthase. Elevated ADMA levels lead to decreased postocclusive vasodilation. Elevated ADMA has been shown in previous studies in children with IBD, type 1 diabetes mellitus and familial hypercholestero-lemia [59]. Clinical and experimental data suggest that there is a multifaceted link between ADMA and insulin metabolism [60].

E-selectin is a membrane glycoprotein found on the surface of activated ECs and is responsible for leukocyte adhesion. It belongs to the adhesion molecules as well as VCAM-1 and ICAM-1. ICAM-1 is a general marker of a proinflammatory state, an independent predictor of peripheral artery disease and coronary heart disease. VCAM-1 is responsible for the transfer of monocytes to atherosclerotic sites. As a result of cytokine stimulation, increased expression of adhesive molecules in ECs leads to microcirculation disorders with subsequent activation of immune-inflammatory processes [61]. Selectins are involved in the initial phase of leukocyte adhesion at sites of inflammation or injury, while the intercellular adhesion molecule mediates the tight attachment of leukocytes and platelets to the endothelium [62]. Levels of soluble adhesion molecules reflect this process of vascular wall inflammation and have been found to predict cardiovascular events in healthy populations [63-65]. Biomarker levels are independent of gender, as shown by Ho et al., in the marker VCAM-1, in the hsCRP marker, and, in the ADMA marker [67-69].

Nowak et al., confirmed higher VCAM-1 levels in CF patients compared to healthy controls, whereas there was no difference in platelet (P)-selectin levels [70]. VCAM-1 is a ligand of very late antigen 4, which is involved in the adhesion of leukocytes to the endothelium [71]. Its levels were higher in coronary artery disease and hypertension, obesity and diabetes mellitus, as well as in women with preeclampsia [57,72-74]. In patients with renal insufficiency without diabetes and atherosclerosis, VCAM-1 correlated with carotid intima-media thickness [75]. A six-month follow-up of 75 patients with acute coronary syndrome showed that VCAM-1 levels predicted the risk of future serious cardiac events [76].

Contrary to the claim that soluble VCAM-1 is an independent risk factor for cardiovascular events, both soluble and membrane VCAM-1 are non-specific [77]. VCAM-1 overexpression occurs in acute respiratory distress syndrome, breast cancer and non-small cell lung cancer, and rheumatoid arthritis, where VCAM-1 levels decline after treatment with infliximab and methotrexate [78-82]. The significance of the available results of this molecule is mainly clinical. Overall, VCAM-1 can be considered a risk factor for CVD in patients with severe endothelial damage and in others as a marker of AS progression. De Rose et al., did not show a difference in VCAM-1 levels in CF patients compared to a control group of healthy individuals [83].

Strong evidence suggests that many different disease processes and risk factors including chronic inflammatory, autoimmune and widespread systemic diseases, such as pulmonary diseases and sleep-disordered breathing, rheumatic diseases, celiac disease, diabetes mellitus, inflammatory bowel diseases, chronic kidney diseases, depression diseases, autoimmune thyroiditis, infection, sepsis, hemolytic-uremic syndrome, Henoch-Schonlein purpura, Kawasaki disease, low birth weight, leukemia, cancer, beta-thalassemia major, and others, contribute significantly to a high risk of ED and premature changes in the vessel wall leading to CVD [31].

ED in Human Diseases and Clinical Events

In recent years, a large body of literature has been published on the impairment of endothelium-dependent vasodilation in several groups of patients. Overall, these results support the existence of a link between ED and the probability of developing structural changes in circulation. Studies completed by us and others support

a link between ED and the development of various human disease conditions in a number of ways, thus the presence of ED has been implicated in the pathogenesis of various conditions and for the induction of proatherothrombotic mechanisms. However, available data suggest that it remains to be determined how individual mechanisms contribute to the activation of these processes. This is a critical question for targeting preventive strategies in clinical practice in subjects with specific risk factors, thus advances in our understanding of ED in a myriad of diseases further will lead to targeted therapies and specific treatment strategies.

In the following section, we provide an overview of major individual diseases.

Study Groups

Pulmonary Diseases

Pulmonary diseases associated with increased CVD risk include especially asthma, chronic obstructive pulmonary disease, cystic fibrosis, alpha1-antitrypsin deficiency, obstructive sleep apnea.

Asthma

Asthma is a common chronic inflammatory disease of the airways of the lungs, characterized by recurrent reversible airflow obstruction and airway hyper-responsiveness in the presence of typical symptoms such as wheezing, shortness of breath and dry cough [84]. The airways of asthma patients are characterized by microvascular hyperpermeability, an increased cross-sectional submucosal vascular, an increased number of vessels and intimal thickening [85]. The stimulus for new vessel formation in asthma is still unclear; proangiogenic activity and endothelial growth factors have been investigated by Kanazawa et al., and Simcock et al., [86-87]. Asthma patients also demonstrate increased Vascular Endothelial Growth Factor (VEGF), similar to chronic bronchitis patients. VEGF is a highly specific growth factor for ECs that is produced in response to hypoxia and induces cell proliferation, migration and prevents endothelial cell apoptosis.

ED is associated with asthma and worsens with disease progression [88-89]. Cortez e Castro et al., highlights NO gene polymorphisms and angiotensin converting enzyme (ACE) as a crucial element in asthma ED and vascular aging, and addresses the mapping of genetic susceptibility to asthma ED [90]. In recent years, a strong correlation between neovascularization and asthma severity has been confirmed as well as impact of profibrotic mediators and ED markers to pulmonary function in patients with uncontrolled moderate asthma Makieieva et al.,confirmed ED in asthma children as well as dependence of asthma severity on functional state of the vascular endothelium [91-92].

The pathophysiological mechanisms of premature manifestation of CVD in asthma have not yet been fully elucidated; chronic systemic inflammation plays a key role. Asthma, as well as COPD are associated with increased inflammation and increased cardiovascular events [93]. Severe asthma is associated with massively increased mucus production and neutrophilic airway inflammation. The evidence for ED in asthma is not as great as that in COPD; however, Yildiz et al., demonstrated reduced FMD levels in asthma patients and relation of FMD to asthma severity that suggests worsening of endothelial function with disease progression similarly to COPD [89]. Chronic inflammation can increase arterial stiffness, a risk factor for CVD, increasing with disease severity. Increased arterial stiffness, expressed as pulse wave velocity of carotid and femur, was examined and confirmed in children with mild to moderate asthma by Steinmann et al., also confirmed inflammatory arterial changes in asthma [94-95]. Lee et al., and Liu et al., demonstrated a higher risk of IHD and a gender effect in asthmatics [96-97]. Women are at a higher risk of CVD in asthma as well as in other inflammatory immune diseases, such as IBD, rheumatoid arthritis due to estrogen influence and generally higher BMI [98-99]. Impaired lung function is another risk factor for the premature manifestation of CVD [100].

The incidence of asthma is steadily increasing; approximately 300 million people suffer from asthma worldwide. Wanner and Mendes, confirmed structural changes in childhood in atopic children and glucocorticosteroid effect for endothelium-dependent vasodilation regeneration [101]. Asthma is the most common chronic disease in children, however, unlike many studies in adults, there is insufficient evidence of ED in children. As the author's yet unpublished study shows on a group of 52 young adults with asthma, a decrease in RHI combined with elevated hsCRP suggests ED in asthma. Salonen et al., demonstrated an inverse correlation of fractional exhaled NO (FENO) in patients with IHD to some risk markers of AS, such as plasma levels of triglycerides and glycated haemoglobin (HbA1c), and to diseases leading secondarily to AS progression, but without evidence of inflammatory or other biomarkers, most likely on the basis of ED with consequent decreased NO production and increased NO degradation in hyperglycaemia and higher triglycerides concentrations [102]. The positive effect of inhaled corticosteroids (ICS) was demonstrated by the following studies: reduction of vascularity in bronchial biopsy specimens and reduction of VEGF levels also after 6 months of ICS treatment. These findings suggest the key role of inflammation of the increased vascularity and vascular remodeling in asthma. Shoda et al., described the importance of ECs in the development and exacerbation of allergic disorders, and confirmed TNF-α-associated angiogenesis, leukocyte adhesion, IL-33-mediated responses, periostin, thymus and activation-regulated chemokine (TARC) production as part of corticosteroid-refractory endothelial cell responses and functions [103]. These corticosteroid-refractory reactions may be involved in the refractory processes of allergic disorders, and endothelial-targeted therapy may thus become a treatment option for corticosteroid-refractory allergic disorders.

Chronic Obstructive Pulmonary Disease (COPD)

COPD is a heterogeneous disorder with several phenotypes in 6% -8% of the population, a multisystemic inflammatory lung disease that is caused mostly by cigarette smoking, and is associated with a significantly higher risk of CVD. CVD plays an important role in morbidity and mortality in patients with COPD [104]. 20% of COPD patients have coexisting CVD, mainly in patients with severe airway obstruction who have more than a twofold increased risk of CVD which is associated with an increased number of hospitalization as well as mortality [104-105]. Airway obstruction is a significant independent predictor of cardiovascular events. In COPD, the association between airflow obstruction and impaired endothelial function is modified by physical activity [106].

Therefore, it is important to early identify patients at high risk of CVD to initiate prevention strategies. COPD is the fourth leading cause of mortality with more than 3 million deaths per year. In COPD as well as in severe asthma and other hypersecretive diseases such as cystic fibrosis, neutrophilic airways inflammation is a key disease feature. In recent years, the impaired endothelium has been proven to be a key factor in the pathogenesis of emphysema, the most characteristic pathological sign of COPD [107]. In COPD, ED is associated with forced expiratory volume

in 1 sec (FEV1) and emphysema, and is related to prognosis [108]. Hancox et al., demonstrated the relationship of lung function with ED and sex differences; lower lung volumes and airway obstruction were associated with ED among women, weaker in men. These findings confirmed the increased risk of CVD among people with poor lung function and suggest the existing gender differences in ED. There is evidence of the presence of ED in early COPD where it is already detected in the pulmonary arteries [109]. Transendothelial migration (TEM) is a mechanism by which the endothelium may play a role in COPD and asthma [84]. Serum levels of ICAM-1 are inversely related to lung function and are also associated with increased percentages of emphysema on computed tomography (CT) scan [1, 110]. The increase ICAM-1 levels might be related to the increase in inflammation in COPD [111]. Peinado et al., described structural and functional injury of the small pulmonary arteries which is detectable in the early phase of COPD, such as vessel wall thickening, ED, vascular smooth muscle proliferation and inflammatory cell infiltration [112]. During the progression of COPD, apoptosis may occur in ECs and it could be visualized on CT where the peripheral vasculature of the lungs is reduced and remodeled [113-115]. The common results of these pathophysiological processes are related to the development of pulmonary hypertension and right ventricular dysfunction - cor pulmonale [116]. The development of CVD, like right ventricular dysfunction, pulmonary hypertension, coronary artery disease, and atherosclerosis, is described by Falk et al., [117]. Penaido et al., confirmed the structural alterations of the intimal layer in small pulmonary arteries as an early feature in COPD and that cigarette smoke plays the main role in the pathogenesis of structural and functional alterations of the pulmonary vasculature in COPD [118].

Thomashow et al., confirmed the main role of endothelial cell apoptosis in the pathogenesis of emphysema [119]. Endothelial microparticles (MPs) are membrane vesicles that are shed by activated or apoptotic ECs [120]. ED of systemic arteries, as assessed by FMD, is also associated with increased levels of endothelial MPs and with emphysema [121-122]. Endothelial MPs levels are increased in patients with frequent exacerbations, which contribute to the increased incidence of cardiovascular events after COPD exacerbation and also may predict patients with rapid FEV1 decline [123-125]. Thomashow et al., confirmed a positive correlation of MPs with the severity of emphysema in COPD patients and evaluated the number of circulating endothelial MPs as a marker of endothelial disorder in patients with COPD and their relationship to the measurement of emphysema [119]. Acute COPD exacerbation is associated with worsening endothelial function [126]. ED occurs in the early stages of pulmonary artery hypertension (PAH), and likely contributes to the progression. Endothelial injury is followed by vasoconstriction and pulmonary arteries remodeling. Clinical data proved the development of emphysema and PAH by ED [127]. PAH in COPD is driven by hypoxic vasoconstriction, systemic inflammation, ED, as well as chronic lung inflammation and impaired lung function. Reduction in lung function is an independent risk factor for CVD [128]. Other studies confirming ED in COPD exist [129,88]. In the Emphysema and Cancer Action Project (EMCP) study, ED measured by FMD was significantly related to gas transfer, lower FEV1 and the percentage of emphysema seen on CT scan in former smokers, and was in relationship to the level of circulating endothelial MPs in the blood, which are markers of both ED and endothelial apoptosis [121]. Although smoking is considered a risk factor for both AS and COPD, it appears that the increased risk of CVD in patients with COPD depends only partially on smoking, and smokers susceptible to COPD may also be prone to lung cancer [130]. It follows that the mechanisms of CVD development in COPD patients are still not fully elucidated, but hypoxia [131], systemic inflammation, oxidative stress, sympathetic activation and physical inactivity are among the key risk factors leading to vascular wall damage and atherosclerotic plaques formation [131-135]. Physical inactivity is not only a disease manifestation of COPD, but also seems to play an important role in COPD progression and the development of comorbidities [136]. Hypoxia has been shown to be associated with pro-atherogenic processes, including systemic inflammation, oxidative stress and an increase in blood pressure [137]. However, its effect on the deterioration of the endothelial function in COPD patients has not been confirmed by Clarenbach et al., On the contrary, hypercapnia showed a relationship with reduced FMD [106].

Insulin resistance may also play a role in ED development in COPD patients. Patients with worse glucose control had worse endothelial function by Urban study and other studies demonstrated that uncontrolled diabetes mellitus is associated with worse lung function in COPD [138-139]. In summary, there is evidence that in COPD patients with the subtype of emphysema apoptosis of the endothelium may result in alveolar destruction and reduced gas transfer. In contrast, in patients with asthma or chronic bronchitis increased VEGF and vascular remodeling in the airways may have a more important role. It is well known that microvasculature dysfunction and increased cardiovascular mortality are associated with COPD; however, it is still not clear whether abnormal endothelium drives COPD or emphysema pathology or if correcting ED has therapeutic potential. Hisata et al., confirmed the key role of endothelial cells; targeting endothelial cell biology using regenerative methods or inhibiting the leucine-rich α-2-glycoprotein-1 pathway may represent a huge therapeutic potential [140].

Smoking and ED

Smokers and early-stage COPD patients show evidence of ED [141]. It is well known that exposure to cigarette smoke damages ECs, and as well as air pollution may activate immune cells, mainly macrophages and neutrophils which drive ROS production and systemic inflammation [116]. In smokers and COPD patients, small arteries are amplified [142]. The relationship between COPD and ED cannot be explained by smoking alone; however, decreased soluble receptor levels for advanced glycation and products (sRAGE) in COPD patients may explain the relationship between COPD and higher CVD risk. sRAGE have antiatherogenic properties and are significantly positively associated with FMD levels in COPD patients [143].

Cystic Fibrosis (CF)

CF is a progressive chronic inflammatory disease with multiple system involvement, caused by CF transmembrane conductance regulator (CFTR) mutations, leading to disruption of ion transport in the chloride channel with consequently decreased chloride secretion, increased sodium reabsorption and viscous mucus production with impaired mucociliary clearance. However, a higher risk of CVD in CF has not been clearly identified [144]. CTFR is an anion channel expressed in both epithelial and endothelial cells, and it is also involved in the transport of sphingosine-1 phosphate, a vascular barrier-enhancing sphingolipidm [145-146].

Endothelium in CF can lead to excessive angiogenesis, pulmonary and portal hypertension, CF-related diabetes, and a higher risk of CVD [147]. It is confirmed that ECs express functional CFTR protein which primarily acts as a chloride channel. Despite the important role of ECs, some endothelial functions in CF still remain under-investigated. The pathophysiology of CVD in CF

patients has been described by Reverri et al., as the gradual ED development from localized inflammatory airway process to systemic vascular dysfunction, with emphasis on the relationship between colonization, systemic inflammatory response and oxidative stress [148]. The initial CFTR dysregulation is followed by impaired mucociliary airway clearance, chronic respiratory infection with subsequent persistent pro-inflammatory activity with oxidative stress, acute exacerbations with progressive lung destruction and/or ED with subsequent development of CVD. CF progression is responsible for chronic airway infection and especially acute exacerbation with activation of inflammatory immune processes with massive infiltration of polymorphonuclear neutrophils into the airways, followed by an overproduction of proinflammatory cytokines and ROS. Several studies support hypothesis that airway colonization might interfere with microvascular function.

Due to great treatment options of CFTR modulators in recent years, the quality and life expectancy of patients has continuously improved. With the prolonged life expectancy of CF patients, CF can be expected to be co-morbid in adults, compounded by a chronic systemic inflammatory process in a combination with a prothrombotic state and oxidative stress [144]. What definitely causes the risk of CVD in CF is not fully understood. In general, obesity is considered to be the main CVD risk factor, but in adult CF patients, nutritional status is usually poorer in comparison with the general population. The main risk factors for CVD development in CF include the systemic inflammatory process, oxidative stress, prothrombogenic factors, dyslipidemia - especially lower HDL cholesterol, omega 3 fatty acids, increased arachidonic acid and cholesterol metabolism disorders, high fat diet, ED, lack of physical activity, diabetes and gender-related differences in sex hormones [149-150]. Pancreatic insufficiency is also associated with an imbalance of oxidants and antioxidants, leading to a greater susceptibility to a lack of fat-soluble antioxidants and vitamins, despite substituents. Oxidative stress is one of the most researched risk factors in recent years in relation to the higher risk of CVD in CF due to the deteriorating bioavailability of NO leading to ED [151-152]. Oxidative stress plays an essential role in the pathogenesis of lung impairment in CF patients at an early age [153-154]. Tucker et al., described the possibility of improving endothelial function and reducing oxidative stress by using antioxidants in young CF patients, assessing ED by FMD before and two hours after administration of an antioxidant cocktail consisting of vitamin C, E, alpha-lipoic acid with an evaluation of change serum concentrations of α-tocopherol and lipid hydroperoxide [152]. Reverri et al., evaluated biomarkers of inflammation (CRP, IL-6, TNF-α) and oxidative process parameters in relation to azithromycin treatment, which has anti-inflammatory and antimicrobial effects [148]. The study showed elevated inflammatory markers with concomitantly lower vitamin D levels in CF patients, and in azithromycin-treated patients more serious clinical and radiological findings. Azithromycin treatment did not affect oxidative parameters, but led to a decrease in TNF-α levels. Thus, macrolide antibiotics affect the inflammatory process, in particular the production of proinflammatory cytokines, oxidative inflammation in phagocytes and neutrophil migration.

Totani et al., demonstrated ED in relation to disrupted CFTR channel in CF as a possible pathogenetic mechanism, thereby confirming the regulatory function of CFTR in ECs [155]. Poore et al., confirmed evidence of ED in fairly healthy children with CF using FMD of brachial artery, and the relationship of ED to lung function and exercise capacity [156]. He demonstrated ED in young CF patients who had good compensation for the underlying disease, as well as higher levels of the inflammatory activity parameter (hsCRP), which were inversely correlated with lung function. No significant correlation was found between hsCRP levels and ED measurements by FMD.

CVDs in CF patients usually result from the secondary effects of progressive lung disease. With an introduction of CFTR modulator therapy into practice, a further improvement in the quality of life can be expected, as well as its prolongation, and thus an increase in the prevalence of vascular diseases. Due to the localization of CFTR in the vascular endothelium and smooth muscles, CFTR dysfunction can directly affect cardiovascular function [157]. Early noninvasive detection of ED may have therapeutic and prognostic implications in high-risk young CF patients. The reversibility of ED may be a primary target in the effort to optimize therapeutic strategies and to decrease CVD risk in CF. As evidenced by author's study of an evaluation of endothelial function in young CF subjects using a combined diagnostic approach by measuring RHI and ED-related biomarkers, elevated biomarkers in CF children with not yet demonstrated RHI changes but with significantly reduced RHI in adulthood indicate the possible occurrence of ED with CF-related specific risk factors over time [158]. In addition, lipoprotein abnormalities correlate with conclusions previously published studies, lower HDL levels are considered a significant risk factor for atherogenesis in CF [144,156]. Based on these data, it is conceivable that allowing early identification, targeted management and therapeutic correction of ED may lead to improved prognosis in high-risk CF patients.

Obstructive Sleep Apnea (OSA)

OSA is characterized by snoring, apnea, non-quality sleep and excessive daytime sleepiness. OSA is associated with vascular alterations, reduced endothelial repair capacity, and vascular reactivity. Hypoxia-reoxygenation episodes usually occur repeatedly during the night, and are associated with an increased CVD risk, including hypertension, insulin resistance, coronary artery disease, cerebrovascular disease [159-160]. One of the possible mechanisms of ED development in OSA is the cycle of hypoxia and re-oxygenation when chronic intermittent hypoxia and sleep loss may increase the levels of inflammatory markers and oxidative stress, as well as increase procoagulant and prothrombotic activity and create a hemostatic imbalance with reduced NO bioavailability. It is still unclear whether ED in OSA is due to alterations in vasoconstriction mechanisms related to angiotensin II or endothelin 1. In addition, the ability of the endothelial repair to protect against this increased damage is reduced [161]. Jurado-Gamez et al., confirmed in a study of 69 OSA patients a reduced morning ischemic reactive hyperemia and significantly higher plasma levels of malondialdehyde and 8-hydroxydeoxyguanosine due to oxidative stress [162].

Bironneau et al., confirmed that moderate to severe OSA has no impact on digital micro-vascular endothelial function in patients with type 2 diabetes (T2D), on the other hand, discovered an association between increasing OSA severity and higher systolic blood pressure, lower circulating levels of adiponectin and higher levels of P-selectin [163]. Oxidative stress and concomitant systemic inflammation are two mechanisms suggested contributing to the increased CVD risk for both in OSA and T2D. The question is whether OSA therapy can ameliorate endothelial function and thereby reduce cardiovascular risk [164-165]. The studies examined the effect of continuous positive airway pressure (CPAP) therapy, but with conflicting results [118,166]. Schwarz et al.,

noted a significant improvement in endothelial function after CPAP treatment. Linn et al., demonstrated recovered serum NO derivatives and improved endothelial function as measured by FMD in responders to oral appliance therapy [167-168]. Lurie showed that the administration of CPAP may reverse changes associated with ED and so decrease the risk of premature manifestation of CVD in OSA patients [169].

Alpha1-Antitrypsin Deficiency (AATD)

AATD is a rare genetic disease caused by mutations of the SERPINA1 gene. Alpha1-antitrypsin (AAT) reduces circulating concentrations of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8 (Pott et al; Lockett et al., thereby having important anti-inflammatory and immune-modulatory effects [170-171]. The effect of severe AATD on the risk of CVD developing is still unclear. AAT is produced by hepatocytes, as well as by neutrophils and monocyte/macrophages, inhibiting neutrophil elastase, which is released during the inflammatory process and leads to the breakdown of elastase. Patients with AATD have lung elastase destruction leading to emphysema.

Elastin is a major component of the elastic layer of the vessel wall, and the degradation of elastic fibers plays an important role in the loss of vessel tone and the development of AS. These findings indicate the importance of AAT in the atherogenic process. Current studies indicate at least two mechanisms by which AAT may modulate atherogenesis: a direct interaction between AAT and apolipoprotein B on LDL particles and furthermore, an AAT-LDL complex detectable in atherosclerotic lesions in coronary arteries, both were demonstrated by Murat Sumer and Erturk and Redfors et al. [172-173]. Disease progression is associated with variation in AAT, and low AAT levels promote atherogenesis. Talmud et al., confirmed the relationship between angiographically defined progression of coronary artery disease and common AAT variants, 11478G>A, V213A, and the deficiency alleles S and Z in two studies in post-coronary bypass men and in patients with T2D [174].

AATD is associated with the development of COPD similar to lung disease. The comorbidities in patients with AATD-related lung diseases include CVD. In combination with smoking, AATD can cause COPD which is related to a higher cardiovascular risk. The causal relationship between genetic AATD and increased cardiovascular risk is demonstrated by Curjuric et al., who revealed associations of serum AAT and SERPINA1 mutations with carotid IMT. Only a few studies have examined the comorbidity of AATD patients and confirmed an increased risk for aortic stiffness [175]. Fähndrich et al., proved significant differences between AATD and COPD patients, with a lower CVD incidence (hypertension, chronic heart failure, diabetes, cardiac infarction, and cardiac arterial disease) in AATD, so AATD is associated with lower triglyceride and HbA1c levels [176]. The main finding of the Fähndrich study is the confirmation of fewer manifestations of periphery and coronary artery disease in AATD-related lung disease, which highlights the possibility of specific mechanisms in AATD and indicates that AATD likely impacts on processes involved in CVD. Duckers et al., also confirmed an increased aortic stiffness in AATD patients compared to control individuals without COPD [177]. Dichtl et al., confirmed fewer cleaved AAT fragments in atherosclerotic plaques leading to a reduction in atherosclerotic inflammation and IHD risk, suggesting that low serum AAT or the Z mutation may biochemically or genetically protect against IHD [178].

Rheumatic Diseases

Chronic inflammatory and autoimmune states such as systemic lupus erythematosus, rheumatoid arthritis, dermatomyositis and psoriasis have been identified as independent risk factors for early atherosclerosis and CVD. Vasculopathy is an important hallmark of many chronic systemic inflammatory connective tissue diseases.

Systemic Lupus Erythematosus (SLE)

SLE is a multisystemic autoimmune disease characterized by multiorgan damage including renal and neuropsychiatric disability. In addition, SLE is associated with cardiovascular damage. AS is one of the most common causes of mortality and morbidity in patients with SLE. Lupus-related factors such as inflammation, immune dysregulation, renal involvement and glucocorticoid treatment lead to impaired endothelial function, however, traditional cardiovascular risk factors are also present in patients with SLE. Tydén et al., described type I interferon (IFN) system as a key element in the pathogenesis of CVD in SLE and its ability to induce both ED and platelet activation [179]. Oxidative stress, oxidized LDL immune complexes (oxLDL-IC) and pro-inflammatory HDL are some of the most common causes of atherogenesis in SLE patients. Oates et al., determined the association between subclinical accelerated atherosclerosis and oxLDL-IC, as well as increased levels of pro-inflammatory HDL in SLE [180]. OxLDL-IC are associated with accelerated atherosclerotic plaque and ED in SLE patients. Mak et al., confirmed ED by FMD in patients with SLE without history or clinical symptoms of CVD and showed the main risk factors in these patients such as diabetes mellitus, kidney disease and hypertension that potentially contribute to ED [181]. Tydén et al., confirmed the association between low plasma concentrations of apolipoprotein M (apoM) and disease activity as well as impaired endothelial function [182]. Plasma apoM levels are thought to be low due to chronic inflammation. ApoM is important for normal endothelial function and appears to be a new marker of endothelial function in young patients with SLE.

Rheumatoid Arthritis (RA)

RA is a chronic inflammatory autoimmune disease affecting about 1% of the population, three times more common in women. RA is characterized by synovitis which leads to damage in bones and cartilages in the joints. Extra-articular manifestations, including CVD, are common in RA. ED as a preclinical marker of AS is commonly detected in RA patients. The risk for developing CVD is approximately 50% higher compared to general population [183]. The role of dysfunctional endothelium as a causal mechanism of extraarticular manifestation of RA has been discussed in a number of studies. RA-related inflammation might contribute to the excess CVD risk [184-185]. The systemic inflammatory process leads to ECs activation and increases the expression of leukocyte adhesion molecules, which ultimately leads to AS. ECs activation can be of two types. In the first type, the activation is faster, ECs loosen their junctions and start to interact with leukocytes and platelets. The second type of activation leads to the expression of pro-inflammatory cytokines, such as TNF-alfa and IL-1 [186].

Evidence suggests the important role of ED in increased cardiovascular risk in RA patients. However, the exact pathophysio-logical mechanisms of atherogenesis in RA are still poorly understood. Maiuolo et al., tried to elucidate pathophysiological processes of AS in RA and highlighted a key role of inflammatory cytokines, in particular, TNF-α, synthesized by ECs, which plays a major role in joint destruction and also increases cellular infiltration into the synovium by increasing chemokine expression, activating ECs and increasing angiogenesis

[187]. In addition, TNF-α, in combination with IL-1, can cause bone damage, which is a hallmark of RA. Finally, IL-1 and IL-6 lead to joint destruction and activate disease progression. IL-6 plays a major role in RA because it upregulates chemokines that attract T cells and increase cellular infiltration. In addition, some studies described the possibility of complete protection against RA due to the absence of IL-6. ED plays a key role in the process leading to brain-nerve barrier disruption, which is associated with neurodegenerative disease related to RA. Therefore, maintaining endothelial integrity through lifestyle and nutritional interventions is part of a therapeutic approach in patients with RA.

Systemic Sclerosis (SS)

SS is an autoimmune rheumatic disease affecting connective tissue that leads to fibrosis of various organs and ED with primary microvascular injury. In addition to microvasculature damage, large vessel disease also occurs in SS. Endothelial injury and fibroproliferative vasculopathy of small vessels are pathological hallmarks of SS, as described by Di Martino et al., who investigated the role of circulating ECs as possible biomarkers of SS [188]. Damage to ECs gradually leads to apoptosis with primary involvement of the microvascular system, especially small arteries, arterioles, and capillaries. Due to disease progression and ischemia-reperfusion changes, vascular remodeling occurs with intimate and medial hypertrophy, adventitia fibrosis, and subsequent lumen narrowing. In addition, decreased blood flow and chronic tissue hypoxia, along with impaired angiogenesis and vasculogenesis, cause severe organ damage such as pulmonary arterial hypertension (PAH). Recent data confirmed the key role of ECs dysfunction in disease pathogenesis and showed that ED is present in SS patients even without clinical manifestations and without classic CVD risk factors.

ED pathophysiological processis associated with chronic inflammation and ischemia-reperfusion injury [189]. Especially due to chronic inflammatory process, SS increases the risk of CVD, including PAH, AS, right and left ventricular dysfunction, arrhythmias, conduction defects, pericardial disease, and valvular heart disease. PAH is a late common complication of SS and carries a poor prognosis [190]. Some cardiovascular risk factors such as dyslipidemia, diabetes mellitus and obesity are less common in SS patients compared to RA patients [191]. González-Martín, described the possible vasoprotective effect of vitamin D and on the other hand the connection of corticotherapy with early vascular damage, which can lead to ED [192].

Dermatomyositis (DM)

DM is an autoimmune chronic inflammatory disease of the skin and muscles, causing muscle weakness of proximal upper and lower limbs. ED is associated with serious complications such as skin ulceration, renal, cardiac and pulmonary involvement.

Vasculopathy is an important hallmark of systemic chronic inflammatory connective tissue diseases affecting the skin, mainly SLE, DM and SS, associated with chronic inflammation and leading to an increased risk of CVD. Chronic immune activation appears to be an important factor in pathophysiology of the development of vasculopathic changes. In particular, IFN-driven inflammation is associated with vasculopathy development through direct and indirect angiostatic effects. Type I IFN overexpression occurs in many autoimmune rheumatic diseases. The chronic inflammatory process can disrupt the Angiopoietin-Tie receptor system and the VEGF system; increased VEGF is considered a biomarker of impaired angiogenesis. Soluble adhesive molecules (ICAM-1 and VCAM-1) are considered reliable markers for endothelial activation. Elevated circulating levels of IFN-inducible inflammatory chemokines C-X-C motif ligand (CXCL) 9 and CXCL10, potent chemo-attractants promoting leukocyte recruitment to inflamed tissues, were found in DM. These chemokines may be produced within DM muscle and their overexpression may correlate with vasculopathy severity. Type I IFN can alter vasculature and promote vasculopathy in DM patients, has a direct angiostatic effect on ECs, and also an indirect one through induction of CXCL9 and CXCL10. Type I IFN is associated with ED and a higher risk of CVD. Galectin-9 and CXCL10 are well-known DM-specific biomarkers and high levels of Galectin-9 were considered as the DM-specific biomarker with direct angiostatic effects [193].

The Ekholm study confirmed a decrease in the number and function of circulating progenitor ECs and an increase in type 1 IFN [194]. In a study by Kishi et al, biomarkers of endothelial function, such as circulating ECs, thrombomodulin and vWF, were increased and correlated with extramuscular disease activity in DM patients [195]. however, circulating progenitor ECs were not elevated in the peripheral blood, are produced in the bone marrow, and migrate to blood vessels to differentiate into mature ECs, leading to the formation of new blood vessels, so circulating progenitor ECs correlated inversely with both muscle and disease activity in DM patients. In systemic rheumatic disease, circulating progenitor ECs are often decreased in numbers and function due to vascular damage.

Endothelial injury in systemic autoimmune disorders contributes to morbidity and mortality through accelerated AS, leading to increased CVD risk.

Psoriasis

Psoriasis is a chronic inflammatory skin disease that affects 2-3% of the population and is associated with an increased cardiovascular risk. Patients with psoriasis are at higher risk of atherothrombotic disease independent of traditional cardiovascular risk factors, however, the underlying mechanisms are not fully understood. Inflammation has been shown to be one of the main mechanisms leading to ED. Tawil et al., confirmed a relationship between duration and severity of psoriasis and decreased endothelial vasomotor tone [196]. Simone et al., demonstrated an impaired endothelial function in the cutaneous microvasculature, significantly lower FMD compared to healthy controls, but no significantly different nitroglycerin-induced dilation of the brachial artery in 32 patients with psoriasis [197].

Autoimmune and Chronic Inflammatory (Non-Pulmonary, Non-Rheumatic) Diseases

Diabetes Mellitus

Type 1 diabetes mellitus (T1D) is a chronic autoimmune disease caused by damage of pancreatic islet beta cells (autoimmune insufficiency) or by an idiopathic process, leading to insulin deficiency and the development of the clinical manifestation of diabetes. T1D occurs most often in childhood. The number of pediatric patients with this type of diabetes is growing [198-199]. Patients with diabetes mellitus have a high prevalence of coronary artery disease, as diabetes is implicated in the formation of atherosclerotic plaque. ED is one of the precursor key steps in the development of AS in diabetic individuals [200,60].

Despite intensive insulin therapy, blood glucose levels cannot be kept at normal levels in a large group of children. Due to insufficient insulin levels with a decrease in lipoprotein lipase activity, significantly higher levels of triglycerides, intermediate-density lipoprotein (IDL), and LDL are present. At the same time, low HDL levels are often present. Insulin deficiency is also associated with an increased supply of free fatty acids, as insulin is required to inhibit lipolysis in adipocytes, which in turn leads to stimulation of the liver to higher production of VLDL. There are also changes in the lipoprotein particles with a predominance of cholesterol and triglycerides in individual fractions. The process of atherogenesis is also involved in the non-enzymatic glycation of proteins with the production of advanced glycation products (AGE), which runs on free amino groups and which depends on the plasma half-life of proteins and the degree of hyperglycemia. LDL glycation reduces the classical degradation of LDL via LDL receptors. Subsequently, LDL in plasma accumulates, which leads to alternative degradation by scavenger receptors with the formation of foam cells and atherosclerotic lesions. Glycated LDL particles, especially small LDL, are more prone to oxidation and thus contribute to increased oxidative stress in diabetics. For these reasons, ED often occurs in children with T1D.

Results in the author´s study showed significantly reduced RHI values and elevations of biochemical parameters in patients with T1D compared to healthy controls. These findings suggest a possible incidence of ED in children with T1D, as well as previously published data in studies by Haller et al., and Mahmud et al., . Hurks et al., evaluated endothelial function in children and young adults with T1D using FMD in his study and found significantly reduced FMD levels in patients with T1D who had no clinical problems and were well treated for diabetes [201-203]. Decreased RHI values, and thus possible ED, were already found in children with T1D [46].

Due to the growing number of children with this chronic autoimmune disease, further monitoring of these patients is necessary. Therapeutic approaches including classic agents such as statins, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, antioxidants, and novel agents such as tetrahydrobiopterin, ADMA, and homocysteine, have been used to ameliorate endothelial function of diabetic patients (Tousoulis et al., Cazeau et al., investigated the effect of vitamins C and E on endothelial function [204,60]. This study predicted improvement after the use of antioxidants. However, no significant effect on endothelial function was proven. Oxidative stress, which is induced by hyperglycemia, plays a major role in chronic complications of T1D.

Crohn's Disease

Inflammatory bowel disease (IBD) is a group of chronic, immune-related, relapsing diseases affecting primarily the gastrointestinal tract. IBD consists of two main entities - Crohn's disease (CD) and ulcerative colitis. IBD is characterized by the presence of segmental, granulomatous, transmural inflammation of the intestinal wall. Because of the inflammation of the intestinal wall, complications such as fistulas, abscesses, or stenotic sections can be present. The current conception of CD understands its origin as a disorder in which an abnormal mucosal immune response to the normal intestinal microflora occurs in predisposed individuals [205]. Several studies showed an increased risk of cardiovascular complications in chronic inflammatory disorders, especially during IBD relapses. The endothelium plays a role in the physiologic regulation of vascular tone, cell adhesion, migration, and resistance to thrombosis. Also, ED is associated with an increased risk of atherosclerosis development [206]. There are several potential links between chronic IBD-related inflammatory processes and the risk of CVD, but insight into pathogenetic pathways remains unclear [207].

The study by Mori et al, reveals that a decrease in blood flow during experimental colitis may result in a reduced capacity of colonic arterioles [208]. Altered vascular flow is known to play a role in the pathogenesis and influence the severity of IBD [209].

In author´s study, significantly reduced RHI in CD patients compared to healthy controls were confirmed [210-211]. The finding of significantly lower RHI in children with CD is analogous to the results of previously published studies that evaluated ED in adult patients with IBD using high-resolution ultrasonographic methods. Currently, there are only a few studies about the prevalence of subclinical atherosclerosis in children with CD [3,212]. The results of author´s study show significantly increased levels of ADMA in patients with CD compared to healthy controls. These findings reflect the fact that elevated levels of ADMA, as a competitive inhibitor of NO synthase, lead to a decrease in post-occlusive vasodilation. These results are similar to published data in adults [213]. In author´s study, significantly increased hsCRP levels were found in patients with CD consistent with previously published pediatric data [212]. E-selectin is responsible for leukocyte adhesion in ECs based on immunological-inflammatory interactions. Increased levels of E-selectin were found in children with IBD, the values were comparable to CD patients in author´s study [214].

Chronic Kidney Disease

Chronic kidney disease involves a gradual loss of kidney function. Kidneys filter wastes and excess fluids from the blood, which are then removed to urine. Advanced chronic kidney disease can cause dangerous levels of fluid, electrolytes, and wastes to build up in a body.

Treatment for chronic kidney disease focuses on slowing the progression of kidney damage, usually by controlling the cause. Chronic kidney disease can progress to end-stage kidney failure, which is fatal without dialysis or a kidney transplant.

Chronic kidney disease presents the expression of a medium- and small-size arteriolopathy characterized by intimal hyperplasia, hyalinosis, and smooth muscle cell hypertrophy (nephroangiosclerosis). Such arteriolopathy could reflect systemic dysfunction of the vascular endothelium. It seems to be the basic anatomic disturbances that may eventually lead to disastrous vascular events in the heart, brain, and kidney. Moreover, the small- and medium-size vessels respond inadequately to vasodilatory stimuli, such as acetylcholine, whose production is mediated by NO [215].

Hypertension, stress, inflammation, and uremic toxins are some of the prevalent risk factors of ED in chronic kidney disease. In renal failure, ED and AS are almost universal, as are cardiovascular complications. Endothelial cell damage or injury is associated with clinical conditions such as thrombosis, hypertension, renal failure, and atherosclerosis, and it could also be responsible for accelerated atherosclerosis in patients with chronic renal failure [216].

Inflammation and ED may not only signal but may also causally contribute to loss of renal function in subjects with chronic kidney

failure. Overall, inflammation appears to be a coherent, functional correlation of reduced glomerular filtration rate (GFR) in patients diagnosed with renal disease; and appears to contribute, at least in part, to renal and cardiovascular damage by altering endothelial function. Hemodynamic ED co-occurred with decreased GFR in two studies in patients with moderate-to-severe renal insufficiency and was also relatively well associated with inflammation in the latter study [217-219].

Some possible therapies to improve ED in the kidneys include non-pharmacologic approaches such as smoking cessation, weight reduction, particularly in obese patients with metabolic syndrome or type 2 diabetes mellitus, a low-fat diet, and regular exercise [220].

Celiac Disease

Celiac disease is a systemic autoimmune enteropathy characterized by malabsorption and associated with a higher risk of AS. However, following a gluten-free diet has a beneficial effect on premature AS. Several studies investigated the presence of subclinical AS in patients with celiac disease. De Marchi et al., found a potentially increased risk of early AS in patients with celiac disease compared to healthy individuals, as evidenced by a significant increase in IMT and a significant decrease in FMD and improvement in both on a 6-month gluten-free diet [221]. In addition, significantly elevated levels of both total plasma cholesterol and HDL, as well as decreased CRP, were observed after a gluten-free diet. Patients on a diet have a rate of myocardial infarction and stroke similar to the general population.

An increased risk of IHD and death due to IHD in patients with celiac disease are associated with a chronic inflammatory process and systemic immune activation, both as major pathophysiological mechanisms in celiac disease. Other elements involved in the process of atherogenesis are a decrease in vitamin B and folic acid, which results in an increase in serum levels of homocysteine, a known cardiovascular risk factor. The intestinal microbiota may affect the relationship between celiac disease and its increased AS risk. Modulation of the intestinal microflora by a gluten-free diet can affect atherosclerotic processes in patients with celiac disease [222]. Celiac disease is related to an increased prevalence of dilated cardiomyopathy. Comba et al., showed higher levels of serum adhesion molecules as ED biomarker in patients with newly recognized celiac disease, with a decrease on a fully controlled diet, highlighting the claim that adherence to a gluten-free diet positively affects the levels of vascular adhesion molecules as a reducing CVD risk [223].

Others

Depressive Disorder

Depression is characterized by a depressed mood, loss of interest and appetite, feelings of guilt, disturbed sleep, and low energy. Major depression affects approximately 10% of the population and is associated with an increased risk of CVD. Importantly, the detrimental effect of major depression diseases (MDD) on CVD is already evident in young people [224]. Depression is relatively common in patients with CVD [225]. In patients with IHD, depression is associated with a two to four times increased risk of cardiac mortality compared with patients without depression [226-227]. Currently, the data suggest that the causes of premature CVD manifestations in depressed patients are multifactorial and the pathophysiology is still not fully understood. It is still not clear whether ED is directly connecting depression with cardiovascular risk, or is only a marker of other cardiovascular risk factors [228].

There are probably two pathophysiological pathways of CVD development in MDD. The first one is that depression is associated with dysregulation of the autonomic nervous system and hypothalamic-pituitary-adrenal axis, blood hypercoagulability, and increased inflammation and the second one involves increased cardiovascular risk through vascular ED [225, 229]. Recent studies show that low-grade inflammation and ED are both associated with depressive disorder, independent of lifestyle. Microvascular dysfunction occurs as a result of increased oxidative stress in otherwise healthy young adults with MDD. Both reductions in NO-dependent dilation and changes in vascular smooth muscle function contribute to ED [230]. Depression is associated with higher levels of CRP, IL-6 and TNF-α, and increased expression of monocytes, pro-inflammatory cytokines and chemokines, and therefore may lead to the inflammation that contributes to CVD. Depression may also act as a chronic stressor that contributes to ED through abnormalities in cell adhesion, migration, and proliferation.

Van Dooren et al., declared by evaluation of plasma biomarkers of inflammation (hsCRP, ICAM-1, IL-6, IL-8, TNF-α) and ED (VCAM-1, ICAM-1, E-selectin, vWF) that inflammation and ED are associated with depressive disorder, independent of age, gender, type 2 diabetes mellitus, prior CVD, and lifestyle factors: smoking, alcohol consumption and BMI [231]. The association of inflammation with depressive symptoms is independent of lifestyle factors, however, the association with ED is weakened by smoking, alcohol use and BMI. However, the main known pathophysiological factors are inflammatory pathways, with increased oxidative stress and psychosocial stressors, including acute psychological trauma or early exposure to childhood trauma [232-235]. Psychosocial stressors may stimulate pro-inflammatory cytokines, including increases in IL-6 and TNFα and subsequently lead to the development of depression. In addition, anti-inflammatory treatment has been shown to significantly reduce depressive symptoms in patients with elevated CRP, but not in depressed patients without elevated inflammatory markers [236-237].

Regarding endothelial function, it has been confirmed that depression is associated with impaired FMD of the brachial artery. Two recent studies have reported that FMD was significantly lower in MDD patients compared to healthy individuals [238-239]. Another study by Harris et al., observed depressive symptoms to be inversely related to FMD in healthy postmenopausal women [115].

There is currently no evidence to convincingly demonstrate that the use of antidepressants is associated with improved endothelial function or with decreased cardiovascular risk associated with depression [288]. Dawood found no effect of selective serotonin reuptake inhibitor (SSRI) therapy on the plasma level of ICAM, VCAM, or P-selectin. Baseline arterial plasma noradrenaline levels predicted an improvement in the severity of depression after serotonin-specific reuptake inhibitor treatment [240].

Current data confirm that depression is an independent risk factor associated with a 30% increase in CVD risk [241]. Similarly, patients with bipolar disorder are twice as likely to die from CVD as the general population [242]. Studies in patients with schizophrenia also show an increased risk of CVD [241,243]. According to Morris et al., ED appears to be caused by oxidative stress and inflammation in patients with MDD, bipolar disorder, and schizophrenia [244].

Acute Lymphoblastic Leukemia (ALL)
ALL is a cancer of the lymphoid line of blood cells characterized by the development of large numbers of immature lymphocytes. The symptoms include fatigue, pale skin color, fever, easy bleeding or bruising, enlarged lymph nodes, and bone pain. ALL makes up one-quarter of all childhood malignancies [245]. Sophisticated treatment protocols currently require a better prognosis in children, complete remission is possible in children in more than 85% [246]. However, with the increasing number of patients after successful treatment, ALL goes hand in hand with the increasing number of late side effects of treatment (chemotherapy, radiotherapy) [247]. Anthracycline-induced toxicity can lead to heart failure and dilated cardiomyopathy [248]. In view of this, anthracyclines can also induce changes in the vascular endothelium, and it may be associated with ED and the premature manifestation of AS in children after successful treatment with ALL [8]. Therefore, further monitoring of these children is necessary, as mentioned in previous studies [249-250].

Masopustova et al., confirmed significantly lower RHI values compared to healthy controls as well as a significantly higher value of hsCRP and a tendency to higher values of other ED-related biomarkers in the group of pediatric patients after ALL treatment [251]. The conclusions of this study point to the possible risk of ED and heart disease in patients after treatment of ALL. Giordano's study evaluates endothelial function in pediatric patients with ALL using FMD methods [252]. Similarly, in a study by Sadurska et al., ED has been demonstrated with IMT in patients after treatment with chemotherapy. Two other studies also described ED in children after ALL treatment [253-254. There are also some studies in adults treated for ALL in childhood with similar results [256-257].

Most recent studies focus on the cardiotoxicity of anthracyclines and the prevention of CVD in adults. The cardioprotectants such as Dexrazoxane, Carvedilol, or various nutritional supplements (L-carnitine, Coenzyme Q, etc.) have given contradictory results in the prevention of CVD [258]. To date, no data are available on the effect of these drugs in patients after successful treatment with ALL. According to the available information, only one study has looked at the good effect of home exercise and physical activity on endothelial function in patients treated for ALL [254].

Familial Hypercholesterolemia
Familial hypercholesterolemia is the most serious form of congenital disorders of lipid metabolism from a clinical point of view. It is an autosomal dominant inherited disease, most often caused by mutations in the LDL-receptor gene, and slightly less often by mutations in its ligand-apolipoprotein B 100 gene. Rarely, it may be caused by a specific type of mutation in the subtilisin-kexin type 9 (PCSK9) protein convertase gene. As a result of the above-mentioned mutations, the removal of low-density lipoproteins from the blood is substantially slowed down in hepatocytes, and the level of LDL cholesterol is greatly increased. Very high levels of LDL cholesterol accelerate the development of atherosclerosis. Patients with familial hypercholesterolemia are exposed to very high levels of cholesterol since childhood, therefore may develop CVD at a very young age [259].

Infection
Systemic infection is associated with increased CVD. The infection by various pathogens interacts with the endothelium and may cause altered coagulation, vasculitis and lead to AS [260]. Infectious agents were found in atherosclerotic plaques associated with vascular inflammation [261]. Depending on the pathogen, there are several mechanisms by which the endothelium is altered. The results of current studies suggest that infectious pathogens may damage the arterial wall by a direct viral effect on the endothelium, and also indirectly due to inflammatory cytokines on endothelial NO synthase, as well as lipid modifications of HDL and LDL-cholesterol [262-263].

A recent study has shown that acute infections in children may be associated with impaired endothelium-dependent vasodilation. Charakida et al., found the ED association with even minor infectious illnesses which do not require a doctor's visit or antibiotic therapy in otherwise well children [260].

Viral Infection
Viral infection of ECs is common, such as parainfluenza, adenovirus, herpes simplex virus, poliovirus, echovirus, cytomegalovirus and human immunodeficiency virus (HIV) [264]. Some studies confirmed the relationship between HIV-1 infection and ED and an increased risk of CVD as well as an increased risk of acute coronary syndromes and coronary artery disease compared to the general population by Durand et al., HIV infection may alter the coagulation by heparin cofactor II impairment [265]. In addition to systemic oxidative stress, the HIV virus and its proteins directly alter the oxidative balance in the vascular wall, leading to increased ROS and causing ED [266]. Bush et al., demonstrated an association of antiretroviral therapy with reverse ED in 73% of patients [267].

Sepsis
Sepsis may cause vascular damage via glycocalyx degradation, as well as by impairing FGFR1/EXT1-mediated glycocalyx reconstitution; FGFR1 is a mediator of endothelial repair. Sepsis is characterized by loss of pulmonary EXT1 expression and delayed glycocalyx reconstitution. Accordingly, sepsis-associated degradation of the pulmonary EG contributes to septic lung damage. Yang et al.,demonstrated that FGFR1/EXT1 signaling is necessary for glycocalyx reconstitution and that homeostatic processes are impaired during sepsis [268].

SARS-CoV-2
Endothelium plays a key role in SARS-CoV-2 infection and COVID-19 inflammatory pathologies. SARS-CoV-2 infects airway epithelial cells and also endothelial cells, and it can replicate in ECs. Coronavirus 2019 (COVID-19) has been shown to have increased mortality in people with ED-related conditions such as diabetes, hypertension, and CVD [269]. In addition, the clinical signs of severe COVID-19 disease are similar to vascular dysfunction (multiorgan endothelial damage, thrombosis and angiopathy, dysregulated inflammation, and pulmonary edema) [270-271]. In addition, previous deterioration in vascular function exacerbates the disease due to the fact that COVID-19 common comorbidities such as obesity, hypertension, and diabetes, are all associated with ED. SARS-CoV-2 is able to specifically target and damage endothelium, similarly to emerging viruses, such as henipaviruses, hantaviruses, and highly pathogenic avian influenza viruses [273].

Periodontitis
Periodontitis is the most common oral chronic infectious disease of the tooth that leads to an overall inflammatory reaction and may cause ED and CVD. There are three possible pathways leading to ED: bacteriological, inflammatory and immune. The periodontal biofilm produces an inflammatory response that leads

to progressive destruction of the surrounding tissues. Thus, chronic periodontitis is known as a microbial biofilm-induced disease and thereby leads to an altered vascular response, increased expression of proinflammatory cytokines and adhesion molecules causing ED. Periodontal infection as a possible cause of low-grade systemic inflammation leading to altered vascular function was described by Gurav [274]. Tonetti found an increase in FMD as well as E-selectin levels correlating with intensive care, compared to controls, and therefore declared the benefits of oral health for improving endothelial function. Intensive periodontal therapy can improve impaired vascular endothelial function due to decreased short-term systemic inflammation, also declared that the treatment of severe periodontitis may reverse ED [275-276]. Current data show that treatment of periodontitis can improve endothelial function and thus become an important element in the prevention of CVD [277]. Periodontal infection was identified as a possible cause in the inducement of low-grade systemic inflammation and infection.

Conclusion

Clinical Implications and New Perspectives

Increasing incidence of chronic inflammatory and autoimmune systemic diseases, such as pulmonary and rheumatic diseases, inflammatory bowel diseases, chronic kidney diseases, major depression diseases, celiac disease, diabetes mellitus, infection, cancer, and others, is still rising. Based on current data, the relationship of these disorders to a high risk of endothelial dysfunction and premature atherosclerosis leading to CVD has been confirmed. ED has a clear predictive value for future CVDs.

Prevention

The current goal in CVD prevention is the early identification of patients with an increased risk of premature manifestation of AS. Active search for patients at risk is connected to active search for complications related to ED. Non-invasive testing and monitoring of endothelial functions and a clearly individualized approach based on the principles of personalized medicine are the basis of the current approach to patients at higher risk of CVD. Patients with chronic systemic diseases and CVD comorbidities deserve more intensive monitoring for adverse cardiovascular outcomes.

The evaluation of endothelial dysfunction as a systemic disease deserves much attention from experts with regard to the emerging importance of ED for the control of cardiovascular risk. Assessment of endothelial function appears to be a useful tool for identifying and monitoring at risk patients. Currently, non-invasive measures of endothelial function are of major interest, with the anticipation that patients at risk could be identified early in the absence of clinical symptoms of apparent vascular disease.

In fact, convincing evidence suggests that early detection of AS risk factors may contribute to improving quality of life by delaying the onset of ED as well as reducing the risk of CVD. In this context, new possibility of combined non-invasive diagnostic approach of RHI measurement and laboratory biomarkers examination received a lot of attention due to detecting the preclinical stage of AS in young patients at risk of premature atherosclerosis. New combined diagnostic option has numerous advantages and it could be a suitable method for ED detection and stratification of individual cardiovascular risk in the long-term follow-up of patients at risk of CVD. Further studies are needed to define the role and timing of non-invasive testing and monitoring of endothelial function in patients at high risk of CVD.

In conclusion, a healthy fully functional endothelium is a key element in maintaining cardiovascular homeostasis. Atherosclerosis begins in childhood probably during the first decade of life as a functional clinically asymptomatic endothelial injury and further progresses due to some common and specific risk factors, and manifests itself clinically in adulthood. Especially in pediatric CF patients, the progressive development of microvascular ED from childhood to adulthood was demonstrated by author's study [278]. Early detection of vascular damage allows to reverse ED or to prevent the transition from stable to unstable form of atherosclerotic disease; in summary, to prevent serious cardiovascular events.

Correction of ED can be considered as the main therapeutic strategy for the management of atherosclerosis. Therefore, there is a need to constantly explore and discover new non-invasive possibilities for the early detection of ED, as the fully reversible phase of atherosclerosis, and to search for new and novel ED-related biomarkers. In this context, the function of intestinal microbiota and H_2S has been much discussed in recent years. Considerable evidence suggests their key role in maintaining cardiovascular homeostasis, so intestinal microbiota can be considered a new emerging factor for human health and CVD prevention.

A better understanding of the pathophysiological mechanisms of development of premature AS is essential for future diagnostic and therapeutic strategies. Many chronic and autoimmune diseases have specific risk factors, such as cystic fibrosis and diabetes mellitus, which have been elucidated in recent years and their knowledge can help reduce the development of AS complications and improve quality of life. Furthermore, the life expectancy is extended and life quality of patients is improved also by new therapeutic possibilities of chronic systemic and immune-mediated diseases.

Theraupetic Strategies

Despite the severity of the CVD problem, therapeutic strategies are still limited. Currently, it is desirable to better clarify pathophysiological basis of chronic inflammatory and autoimmune microvascular disease leading to ED, which is a prerequisite for effective pharmacological and non-pharmacological interventions and treatments. Based on recent data, the severity of ED is related to the risk of cardiovascular events. At present, some therapeutic possibilities favorably influencing impaired endothelial function are known. ED reversibility may be the primary goal in an effort to optimize therapeutic strategies and to decrease CVD risk. The endothelium is very plastic and well accessible to therapeutic interventions. Due to research results of COPD and asthma in relation to endothelium, airway endothelial cells may be a new therapeutic target because glucocorticosteroids may regenerate endothelium-dependent vasodilation.

Existing ED therapeutic options include angiotensin-converting enzyme inhibitor, lipid-lowering therapy (statins). The effects of other substances, such as angiotensin-II receptor blockade, TNF-α inhibitor, thromboxane A2 inhibitor, hormone replacement therapy, antioxidants (vitamins C, E, beta-carotene), tetrahydrobiopterin (an essential co-factor for NO production by endothelial NO synthase), homocysteine-lowering drugs (folic acid, pyridoxine), were mostly partial. In recent years, many therapeutic strategies to reverse ED have been studied in patients at risk. The Benefits of improved endothelial function and reduced cardiovascular risk are clearly demonstrated in lipid-lowering, homocysteine-lowering and blood pressure-lowering therapies, highlighting the importance of secondary prevention of atherothrombotic disease.

Angiotensin-converting enzyme inhibitors and angiotensin receptors blockers may increase NO bioavailability by modifying the renin-angiotensin-aldosterone system. Newer therapeutic approaches targeting ED also include insulin sensitizers. In the future, a genetic profile may help to identify potential responders to treatments targeting specific intracellular pathways in vascular endothelium.

Lifestyle Adjustment

Lifestyle adjustment is at the forefront of prevention and early treatment of CVD. Physical activity has been shown to reduce CV risk and to improve endothelial vasomotor function by increasing NO bioavailability even in healthy individuals [1]. Exercise, smoking cessation and dietary measures seem to be the most effective preventive cardiovascular approaches.

Dietary Adjustment

Dietary adjustment including in particular a low-fat diet with high doses of flavonoids and omega-3fatty acid (fresh fruits, vegetables and fish) may improve endothelial function. Both LDL plasma levels and atherosclerotic plaques reduction as an effect of statin therapy lead to a decreased risk of CVD. Based on recent data, a sedentary lifestyle and poor diet are associated with obesity related to ED, increased oxidative stress and proinflammatory markers, leading to AS and CVD. The influence of lifestyle and nutritional intervention aimed at maintaining the integrity of endothelial cells can be considered as a therapeutic option. In conclusion, a healthy lifestyle with sufficient antioxidants and physical activity can improve ED, but further studies are needed.

Namely, we described the clinical units in relation to ED in the review chapter and emphasize the clinical use of endothelial function as a prognostic marker of CVD, although endothelial function is mostly used as a cardiovascular risk indicator. Monitoring of endothelial function plays an irreplaceable role in primary prevention, and risk scoring can be considered in the future to optimize preventive measures in patients at risk of CVD.

In conclusion, a better understanding of the close relationship between the underlying major diseases, ED and early identification of patients at risk, with newly discovered therapeutic options leading to ED recovery could be considered the optimal preventive approach for ED and CVD complications [279-294].

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Chapter 3

Effects of the Hormone Irisin on the Glucose Metabolism

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ABSTRACT

Given its increasing prevalence, obesity is now considered a pandemic. Several bioactive molecules, such as cytokines, acute phase proteins and hormones secreted throughout the body are involved in the development of obesity. The hormones secreted by the white adipose tissue mass that increases with obesity are known to be effective also in the glucose metabolism disorder associated with obesity. In addition, irisin, a muscle-derived hormone that is also secreted by adipose tissue, is considered a significant agent in the prevention/treatment of obesity and the resulting glucose metabolism disorder, with its ability to convert white adipose tissue into brown adipose tissue. In this book chapter we assess the effects of the hormone irisin on the glucose metabolism.

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Introduction

Obesity is a chronic disease involving an increase in body fat mass due to the higher energy intake through food than the energy expended [1]. A long-term high-calorie dietary intake combined with physical inactivity results in obesity and subclinical tissue inflammation. This, in turn, causes an imbalance in glucose metabolism, which leads to insulin resistance and the development of type 2 diabetes mellitus (T2DM) [2,3]. Lifestyle changes, particularly changes in one's diet, physical activity and exercise, remain the best treatment option for obesity. The benefits of exercise have been documented extensively [4]. It has been recently reported that skeletal muscles release certain hormones known as myokines into circulation, especially during or immediately after physical activity, and these can affect the metabolism and alter the production of cytokines in various tissues and organs [5]. After being detected in adults a few years ago via positron emission tomography, human brown adipose tissue (BAT) has been recognized as having the potential to play a role in the treatment of obesity based on the fact that brown adipocytes can dissipate energy in the form of heat, resulting in weight loss. This process occurs via a specific mitochondrial protein called uncoupling protein 1 (UCP1), uncoupling activity of which can be attributed to its ability to transport protons across the inner membrane of mitochondria, inhibit ATP synthesis and dissipate energy as heat [6-8]. The regulation of UCP1 is mainly transcriptional, with the peroxisome proliferator-activated receptor Y coactivator 1α (PGC1α) playing a key role [9]. PGC1α is mainly expressed in BAT, but is also expressed at high levels in skeletal muscle. The expression of PGC1α increases with exercise in mice, rats and humans, while exercise activates AMP-activated protein kinase (AMPK), being the master regulator of cellular metabolism [10,11]. AMPK directly phosphorylates PGC1α. Furthermore, transgenic mice with increased PGC-1α expression in the muscles showed improved metabolic responses, such as age-related obesity and insulin insensitivity [12,13]. An analysis of the adipose tissue in these transgenic mice revealed a significantly increased thermogenic gene program in the subcutaneous adipose tissue, and browning of the adipocytes. These "brite" (white-brown) adipocytes contain high levels of UCP1 mRNAs, and accordingly, the recently discovered hormone irisin is held accountable for these effects [14].

The Hormone Irisin

Irisin is a myokine that is secreted especially after exercise, and is associated with increased energy expenditure by inducing the browning of white adipose tissues [14]. Irisin was first identified in 2012 as a hormone secreted from the muscle cells of transgenic mice with an overexpression of the Ppargc1a gene that encodes PGC1a, which is involved in most metabolic pathways associated with energy metabolism [14]. PGC1a expression stimulates the release of FNDC5 (the long part) and the FNDC5 transmembrane protein, containing 212 amino acids in humans and 209 amino acids in mice and rats [14,15]. The protein sequence includes a signal peptide, a fibronectin type-III domain, a hydrophobic transmembrane domain and a carboxy-terminal domain located in the cytoplasm. After proteolytic cleavage, glycosylation and possibly the dimerization of FNDC5, a new protein that contains the major part of the fibronectin III domain is released. This new protein, containing 112 amino acids and an identical sequence of amino acids in humans and mice, has been named irisin [14,16].

In humans, FNDC5, the precursor of irisin, is expressed at high levels in skeletal muscle, and other muscle-containing organs such as the heart, tongue, and rectum. In contrast, expression of FNDC5 is low in the pancreas and liver, which are key organs involved in glucose homeostasis [17]. Adipose tissue is also an important source of irisin. In rats, irisin has been reported to be secreted from the mature adipocytes of subcutaneous and visceral white adipose tissue, while BAT has been reported to barely express FNDC5 or irisin [18].

Irisin acts systemically on the subcutaneous "beige" fat, and increases expressions of UCP-1 and other thermogenic genes, causing "browning" and regulating energy homeostasis. This suggests that irisin can be used as a target agent in the treatment of metabolic disorders associated with obesity [14,19]. BAT causes increased energy expenditure due to the increased number of mitochondria and high oxygen consumption, and so has therapeutic effects on obesity and obesity-related impaired glucose homeostasis [20]. It is known that after cold exposure, BAT, which increases in mass with cold, has a higher glucose uptake and a greater expression of glucose transporter type 4 (GLUT4), which transports glucose into the cell, than white adipose tissue [21]. Previous studies of irisin levels and FNDC5 expression have reported irisin secreted from adipocytes through FNDC5

expression to be lower in patients with obesity or T2DM than in healthy controls [22,23].

An in vitro study of muscle tissue have shown that the treatment of primary human skeletal muscle cells with irisin (50 nM) for one hour significantly increased intracellular glucose and fatty acid uptake, which concurs somewhat with the results observed after insulin exposure [24]. After six hours of the irisin treatment of these cells, the expression of genes involved in glucose transport and lipid metabolism, such as GLUT4 and PPARA, was increased in myocytes, whereas the expression of genes involved in glycogenolysis or gluconeogenesis was suppressed. Irisin activates the AMPK pathway by reducing intracellular ATP levels. The activation of the AMPK pathway induces the expression of GLUT4 and peroxisome proliferator-activated receptor alpha (PPARA) genes and inhibits the expression of genes involved in glycogenolysis or gluconeogenesis [24]. In another study, recombinant irisin was shown to stimulate glucose uptake after AMPK activation in differentiated L6 muscle cells [25]. The same effects were completely attenuated after the inactivation of the AMPK pathway in the murine C2C12 myoblast cell line when treated with irisin and cultured in high glucose and an increased fatty acid medium, suggesting that irisin uses the AMPK pathway in these effects. In addition, irisin attenuates the palmitic acid-induced inhibition of insulin signaling by inducing AKT and ERK phosphorylation [26].

In vitro studies of liver tissue have revealed the positive effects of irisin [15]. The irisin treatment of insulin-resistant hepatocytes derived from human hepatocellular carcinoma cells suppresses gluconeogenesis by reducing the gene expression of PCK1 (phosphoenolpyruvatecarboxykinase 1) and G6PC (glucose 6 phosphatase) via the phosphoinositide 3-kinase (PI3K)–AKT–FOXO1 pathway, and induces glycogenesis via the PI3K–AKT–glycogen synthase kinase 3 (GSK3) pathway [15,27].

The hormone irisin was first identified reported FNDC5 adenoviral overexpression and a reduction in glucose and insulin levels, indicating an improvement in weight and insulin resistance in high-fat fed mice [14]. Later researchers sought to characterize the role of irisin in glucose regulation, but came up with conflicting results, and the mechanisms have yet to be fully clarified. One study reported decreased levels of fasting glucose and insulin levels after the intraperitoneal administration of recombinant irisin for 14 days to mice fed on a high-fat diet for 10 weeks [19]. Another study reported that irisin-treated diabetic mice fed a high-fat diet developed an improved glucose tolerance, decreased epididymal fat mass, and reduced serum total cholesterol and triglyceride levels when compared to a control group. The improvement in glucose and lipid profile was achieved through the increased glucose uptake associated with decreased gluconeogenesis in the liver [20]. Similarly, in mice with diabetes induced by streptozotocin and a high-fat diet, the subcutaneous administration of irisin reduced fasting blood glucose, and increased insulin sensitivity and hepatic glycogen storage [27]. Furthermore, the overexpression of FNDC5 in obese mice reduced hyperglycemia, hyperinsulinemia, hyperlipidemia and blood pressure, while energy expenditure and lipolysis were increased [21]. It was reported that the intraperitoneal and oral administration of irisin in mice with streptozotocin-induced diabetes reduced glucose levels without affecting weight reported that 16 weeks of irisin treatment did not bring about a change in blood glucose levels in mice with STZ-induced type-1 diabetes mellitus [28,29]. Hou et al. and Zhu et al. also reported a significant improvement in glucose and lipid profiles with recombinant irisin treatment in high-fat diet-fed mice [30,31]. The hormone irisin was found to be increased in the muscles of obese individuals, possibly to maximize the glucose uptake in muscle tissue and prevent hyperglycemia [17,22]. The intraperitoneal injection of irisin into obese and diabetic high-fat diet-fed mice was reported to enhance glucose uptake and accumulation by inducing GLUT4 translocation to the skeletal muscle cell membranes [20]. Only one study involving individuals without T2DM reported an association between a higher night-time energy intake and lower fasting irisin levels the next morning [32]. The decrease in irisin levels in cases of reduced food intake, i.e., energy intake, is believed to be related to the energy and glucose metabolism. The in vivo studies to date investigating the effects of irisin treatments on glucose metabolism have been mostly at a clinical finding level, while pathways through which these effects occur in peripheral tissues have not been fully clarified.

In addition to the interaction between skeletal muscle and adipose tissue, irisin has been reported to play a role in the central nervous system [12,33,34]. The presence of irisin and FNDC5 has also been detected in the nervous system and is expressed in the Purkinje cells of the cerebellum that send projections to the deep cerebellar nuclei and vestibular nuclei [35]. Furthermore, irisin has been shown to be localized in the paraventricular nucleus of the hypothalamus and in the cerebrospinal fluid of humans, suggesting its involvement in metabolic homeostasis through its activity in these regions, being central regions that play a role in the lipid and glucose metabolisms of the organism [36]. Another interesting issue that needs to be addressed has been reported to be the determination of the role of irisin in other brain areas involved in the regulation of energy balance, such as the hypothalamus and brainstem [37]. This is because previous studies involving central irisin administration to mice/rats exploring the central role of irisin did not include an examination of glucose homeostasis. Considering such studies: Zhang et al. examined the effects of recombinant irisin administration to the 3rd ventricle of the brain on blood pressure and cardiac contractibility in rats, Zhang et al. examined the effects of irisin injections into the 3rd ventricle of the brain on locomotor activity in rats, Erden et al. examined the expressions of UCP agents in the hypothalamus, pituitary gland, hippocampus, cerebellum, striatum and cortex of the brain through intracerebroventricular irisin administration in rats, Ferrante et al. examined the effects of intrahypothalamic irisin administration on feeding and orexigenic-anorexigenic peptides, Tekin et al. examined the effects of chronic central irisin administration on feeding behaviors in rats, Tekin et al. examined the effects of chronic central irisin administration on thyroid hormones and energy consumption in rats, Asadi et al. examined the relationship between irisin and ischemic brain injury by administering intracere broventricular irisin to mice, Tekin et al. examined the effects of intracere broventricular irisin administration on the hypothalamus-pituitary-gonadal axis in rats and Siteneski et al. examined the neuroplasticity-related genes and antidepressant effects of central irisin administration in the hippocampus and prefrontal cortex of mice; although it would seem that no research has been conducted on glucose homeostasis [38-48].

Conclusion

Taking into account the above, it is apparent that further studies are needed to explore the effects of central and systemic irisin on the glucose metabolism.

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Chapter 4

Differential Diagnosis of Neuromyelitis Optica Spectrum Disorder

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ABSTRACT

Neuromyelitis Optica Spectrum Disorder is now considered an autoimmune astrocytopathy causing demyelinating and necrotizing disease of the CNS. With many differentials under the rubric of demyelinating disorders affecting the central nervous system, recent research has proven NMOSD to have a distinct pathological and clinical presentation. This also has treatment implications especially in other demyelinating disorders like Multiple Sclerosis wherein certain therapies for MS may prove to be detrimental for patients with NMOSD. Also, patients may present with disorders that have a similar phenotype with NMOSD. Since this includes a host of aetiologies like inflammatory, infective, vascular and malignant diseases, it is imperative for the clinician to rule them out systematically by clinical, serological or radiological features. This review will highlight the common disorders that need to be considered as a differential diagnosis for NMOSD and features that help in their differentiation.

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Keywords: Neuromyelitis Optica Spectrum Disorder, Differential Diagnosis, Demyelination

Introduction

Neuromyelitis Optica Spectrum Disorder is a demyelinating disease limited to the central nervous system. Since its first description by Dr. Eugene Devic in 1894 NMOSD has now come to encompass many clinical presentations though optic neuritis and longitudinally extensive transverse myelitis are the most common [1].

Recent epidemiologic studies have put the prevalence of NMOSD at 10/100,000 in Afro – Caribbean population compared to 1/100,000 among the Caucasian population [2]. Consistently it has been seen that NMOSD has a varying prevalence among different racial groups and is less common in the North American population when compared to the prevalence of another common demyelinating disorder, Multiple Sclerosis. NMOSD characteristically shows a high female preponderance from 3: 1 to 9: 1.Pathophysiologically, this disease is an astrocytopathy which is distinct from other demyelinating disorders like Multiple Sclerosis and MOG associated disorders (oligodendrocytopathy) [2-5]. This has clinical and treatment implications since many disease modifying therapies used in MS have been found to be detrimental in patients with NMOSD (Interferon β and natalizumab). Though with the recent NMOSD criteria (IPND 2015) our diagnosis of this disorder has improved, many differentials may challenge the clinicians especially in seronegative cases. This chapter would aim to highlight the important features that may help to reach our diagnosis in these difficult scenarios [6].

Why Differential Diagnosis of NMOSD Is Important?

The presence of AQP4Ab in the sera of patients with NMOSD is a basis for differentiating patients into seropositive and seronegative group according to the IPND criteria with the seropositive group requiring only one core clinical feature (ON, myelitis, area

postrema syndrome) for diagnosis [6]. However, in patients who are seronegative the clinical and radiological differential diagnosis remains important. Also, it is important to understand that the sensitivity of the AQP4 Ab test depends on the type of assay involved and in certain clinical scenarios as in resource poor settings, this assay may not be readily available. Rather, clinicians may need to identify those patients in whom the pre-test probability would be high and in whom the AQP4 Ab assay needs to be performed. Many diseases including inflammatory, infectious or neoplastic aetiology may involve the CNS and mimic the clinical and radiological phenotypes of patients with NMOSD. Table 1 lists some of the common differentials of this disorder.

Table 1: Common Differentials to Be Considered in Patients with NMOSD

Demyelinating Disorders	Multiple Sclerosis Acute disseminated encephalomyelitis Anti MOG associated disease
Inflammatory	Sarcoidosis Sjogren's disease Systemic lupus erythematosus Neuro- Behcet disease
Infections	Neurosyphilis Herpes virus HTLV1 Borrelia burgdorferi
Vascular disorders	Spinal arteriovenous dural fistula
Malignancy	CNS Lymphoma

Multiple Sclerosis

MS especially during the relapsing course may present very similar to NMOSD. Though there were initial debates whether these two disorders are fundamentally different (NMO was initially described as opticospinal MS) after the discovery of the AQP4 Ab, subsequent studies have confirmed that these disorders are distinct in their epidemiology, pathology, response to treatment and prognosis. Differentiating MS from NMOSD is important since many DMT's used in treatment of MS (Interferon B, fingolimod, natalizumab and alemtuzumab) may even aggravate NMOSD [7-10].

Distinctive characterised of MS from NMOSD are summarised in table 2.

Table 2: Features Differentiating Multiple Sclerosis from Neuromyelitis Optica Spectrum Disorder

	MS	**NMOSD**
Epidemiology Prevalence	More common in north American and European countries Putative role of environmental factors… Moderate female predominance	No role of environmental factors High female preponderance (9:1 – 3:1)
Clinical Features Optic neuritis	 Visual field defect other than Cecocentral scotoma uncommon Severe visual loss uncommon (4.2% in 11 years of disease onset)	 Severe field defects like complete blindness, altitudinal hemianopsia may be seen in up to 25% patients Common (50% in 10 years of disease onset)
Myelitis Brain	Severe myelitis causing complete paresis is rare Painful tonic spasms are rare Area postrema symptoms (intractable hiccups and nausea) are rare	Relatively common (30-70% at first attack) Relatively common (up to 25%) Relatively common

MRI Findings		
Spinal cord	Usually short segment myelitis Except in later stages of the disease	Long segment myelitis (>3 vertebral segments) seen in up to 94% adults
Brain	In axial images, spinal lesions appear asymmetrical and peripheral, often posterior involvement	Central grey matter involvement
	Cloud like enhancement uncommon	Common
	Callosal lesions are small, isolated and non-oedematous	Callosal lesions can be large and oedematous
Optic nerve	Lesions perpendicular to a lateral ventricle	Lesions along the margins of the lateral ventricles Rare
	Cortical and juxta cortical lesions common Optic nerve involvement is anterior pre dominant	Optic chiasmal involvement is relatively common (25-75%)
Cerebrospinal fluid study	Pleocytosis mild to moderate	May be severe (up to 1000/cumm)
	Oligoclonal bands may be present in most patients (up to 97%) and most importantly rarely disappear in follow up	Can be present in some patients (33-43%), mostly disappear in follow up
Optical coherence tomography	Retinal fibre layer thickness reduction is mostly in temporal quadrant	Thickness reduction is mostly in superior/ inferior quadrant

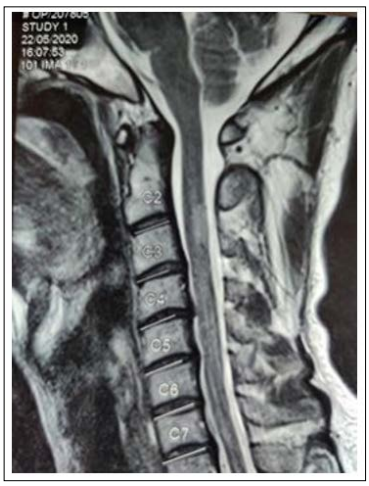

Figure 1: MRI T2w Cervical Spine in A Patient of MS

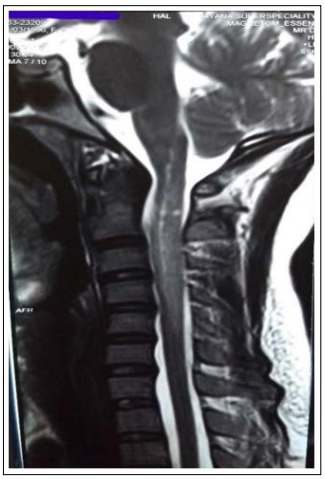

Figure 2: MRI T2W Cervical spine in a patient of NMOSD. Note the long segment myelitis along with extension into dorsal medulla and cord edema which is not seen in MS.

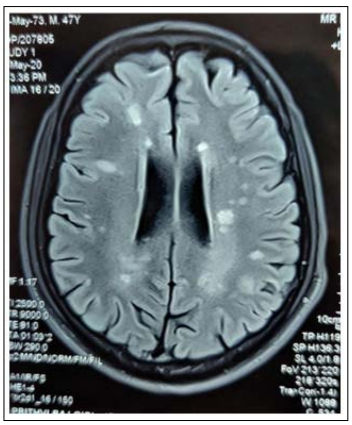

Figure 3: MRI Brain T2 Flair images in a patient of MS. Note the juxtacortical and periventricular plaques which are sharply demarcated

Acute Disseminated Encephalomyelitis

ADEM is another inflammatory disease of the CNS that may mimic clinical presentation with NMOSD especially in paediatric patients. However, some important clinical caveats include a usual polyfocal neurological symptoms at onset, mostly a monophasic course (NMOSD has a relapsing form most commonly) and encephalopathy (manifesting as alteration in consciousness or behavioural change) in ADEM. In NMOSD symptomatic cerebral syndrome as a presenting feature is seen in very few patients (about 8%) [11]. Another important feature of ADEM distinguishing it from NMOSD is that most patients with ADEM have preceding infection (up to 61%) or vaccination (up to 4%) within 4 weeks of onset neurological symptoms [12].

A study by Zhang L et al. demonstrated T2 hyperintense lesions in putamen to favour a diagnosis of ADEM in contrast to hypothalamus involvement in NMOSD [13]. Classically the lesions in ADEM are larger, confluent with uniform enhancement of all the lesions [14]. However, care should be taken when applying these features

in clinical practice since enhancing lesions in MRI can be found only in some patients with ADEM (30-66%) and some patients with ADEM may have a multiphasic course [15].

Myelin Oligodendrocyte Associated Disease (MOGAD)

The inflammatory disease associated with MOG Ab can manifest as a phenotype of NMOSD especially in the seronegative group. While no phenotype is restricted to any specific age group, MOGAD phenotype in both children and adults may have some generalizations. In children less than 11 years of age, ADEM like presentations (encephalopathy, multifocal neurological deficits, large or "fluffy" supratentorial cerebral lesions bilaterally) are common while in adults focal involvement of optic neuritis or longitudinally extensive transverse myelitis are more prevalent [16]. In MOGAD the majority of children are not prone to frequent relapses although the high rate of monophasic disease may be an overestimate.

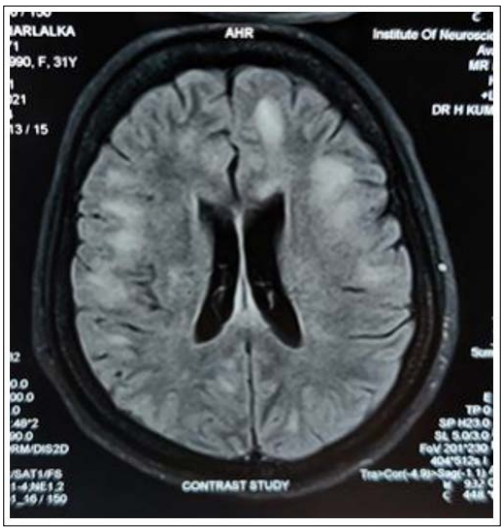

Figure 4: MRI Brain T2 Flair images in a patient with ADEM. Note the large, fluffy, multifocal (compared to smaller and sharply contoured plaques of MS as in Figure 3) lesions and absence of any periventricular signal changes

At the onset, vision loss is often severe and up to 80% patients have bilateral optic nerve involvement similar to NMOSD. Despite the severity of vision loss, recovery is usually good especially in children. Optic disc edema is rare in both MS and NMOSD but is present in up to 86% of MOGAD patients [17-19]. Over 50% of adults with MOGAD have a recurrence of ON which may be the only manifestation of the disease. MOG associated transverse myelitis can affect any segment of the spinal cord but has a greater predilection for conus medullaris (11-41% patients) [20-21].

Radiologically, "optic peri neuritis" or inflammation of the optic sheath and surrounding structures on MRI is a characteristic feature of MOGAD seen in around 50% cases [22-24]. This perineural involvement is a feature that may help differentiate MOGAD from NMOSD or MS. Spinal cord lesions in MOGAD are much less likely to show gadolinium enhancement in the acute phase as compared to NMOSD (26% of MOG patients vs 78% of seropositive NMOSD patients according to one study) [20]. Also, MOG disease affects both grey and white matter of the cord producing linear hyperintensity of the central cord ("pseudo dilatation") or H shaped hyperintensity involving the anterior and posterior horns ("H" sign) on axial sections.

Sarcoidosis

Sarcoidosis is a systemic granulomatous disease that commonly involves the lymph node, skin, lungs eye and only in 5-15% of patients, the nervous system. Most patients with neurosarcoidosis can have systemic manifestations such as hilar adenopathy on chest radiography, erythema nodosum, uveitis, or macular skin lesions along with neurological manifestations. Isolated neurosarcoidosis may require histopathologic evaluation of CNS tissue for confirmation. Radiologically, longitudinally extensive transverse myelitis in sarcoidosis may have subpial gadolinium enhancement and persistent contrast enhancement (> 2 months) though these features are not always present.

Sjogren's Syndrome

Patients with longitudinally extensive transverse myelitis associated with Sjogren's Syndrome meet the diagnostic criteria for NMOSD or are tested positive for the AQP4 Ab in most circumstances. The clinical and radiological features in these patients do not differ from those with NMOSD without Sjogren's Syndrome. The CNS involvement in Sjogren's Syndrome seem to be manifestations of coexisting NMOSD rather than direct involvement due to Sjogren's Syndrome perse.

Systemic Lupus Erythematosus

Most patients with CNS lupus manifest with multifocal complaints such as headache (54%), seizure (42%), hemiparesis (24%) or memory impairment (24%) [25]. Whereas headache, seizure or memory impairment are not seen in NMOSD, some patients with NMOSD may have ANA positive or vice versa. Patients of NMOSD with ANA positivity are treated with immunosuppressives according to guidelines for neuromyelitis optica.

CNS Lymphoma

Primary CNS lymphoma can have many radiological similarities with NMOSD. Both the disorders can have longitudinally extensive transverse myelitis and about 40% of the patients with spinal cord involvement in lymphoma can have intramedullary signal changes without cord swelling thus mimicking a non-malignant etiology. The brain MRI lesions of primary CNS lymphomas while commonly involving the deep grey matter or grey white matter junctions, can have a varied presentation. Also, it is important to remember that treatment with corticosteroids can initially lead to an improvement in the clinical as well as radiological features in primary CNS lymphoma.

To differentiate between CNS lymphoma and NMOSD, tests such as CSF cytology (sensitivity of 2-32%), immunoglobulin heavy chain (IgH) rearrangement testing (sensitivity of 58% and specificity of 85%) or positron emission tomography (PET) can be important tests [26]. However, histopathological examination of the tissue obtained by stereotactic or navigation guided needle biopsy are required for diagnostic confirmation of CNS lymphoma.

Patients with LETM should usually be suspected to have primary CNS lymphoma if:

- Progressive course, with or without partial initial improvement, after treatment with methylprednisolone and/or plasmapheresis
- Lesions show persistent contrast enhancement after 3 months of onset
- Lesions are hypermetabolic on FDG-PET

Neuro- Behcet's Disease

Behcet's disease presents with painful mucocutaneous lesions with diverse systemic involvement. It is more common in the Middle East and regions around the Pacific Rim [27- 28]. CNS involvement of neuro behcet's can be either parenchymal (multifocal/diffuse, brainstem, spinal cord, cerebral or optic nerve) or non-parenchymal (cerebral venous thrombosis, intracranial aneurysms, meningitis). Behcet's disease involves long segment myelitis in a similar presentation to NMOSD.

Since neurological manifestations of Behcet's disease can sometimes precede its systemic manifestations, two levels of diagnostic criteria for Neuro Behcet's have been proposed (definite and probable) in those without systemic manifestations [29].

The salient features of Neuro Behcet's that distinguishes it from NMOSD are presence of headache with or without meningo encephalitis, (about 70%), a progressive course (38%) and severe brainstem or cerebral atrophy [30-31].

Spinal Dural Arterio Venous Fistula

Spinal dural arterio venous fistula (SDAVF) is the most common type of vascular malformation affecting the spinal cord.

It is more common in males in their fifth or sixth decades in contrast to NMOSD which predominates in females and has a wide range of age distribution of presentation [32]. Patients with SDAVF mostly present with a subacute onset progressive myelopathy with acute deteriorations precipitated by exercise or prolonged rest.

The MRI spine in SDAVF presents with LETM very similar to NMOSD. But, SDAVF is characterized by flow voids on T2 weighted images in MRI caused by abnormal dilated intradural veins of the spinal cord. Also, serpentine enhancing vascular structures on T1 weighted contrast image on the dorsal surface of the cord may also be observed. However, it is to be remembered that these classical findings may not always be present in conventional MRI mainly in the early stages of the disease.

Though spinal angiography is recommended in the diagnosis of SDAVF, catheter based angiography remains the procedure of choice for diagnosis.

Infections

Neurosyphilis

Syphilis is a sexually transmitted infectious disease caused by the spirochete bacterium Treponema pallidum. Neurological manifestations of syphilis are usually seen in the tertiary stage of the disease.

Though uncommon, neurosyphilis may cause painful bilateral severe vision loss. This can present in any of the four stages of the disease and can mimic the ON caused by NMOSD. However, in the early stages, ON related to neurosyphilis manifests more as a peri neuritis per se, producing constricted visual fields with normal central vision [33-34]. This pattern when present is very atypical for NMOSD related ON and should alert the clinician for an alternative diagnosis.

Also, bilateral ON with poor response to steroids, HIV infection or positive serum treponemal test warrant further investigations for neurosyphilis. Longitudinally extensive transverse myelitis is a very rare manifestation of syphilis.

Apart from syphilis, various other infections such as herpes virus (Herpes simplex, Epstein Barr virus and cytomegalovirus) dengue virus, human T lymphocyte virus 1 (HTLV1), borrelia burgdorferi (Lyme disease), tuberculosis and Mycoplasma pneumoniae can present with long segment transverse myelitis and/or ON. This becomes particularly challenging since some patients with NMOSD may have CSF pleocytosis. The main differentiating points include presence of systemic signs and absence of serum AQP4 Ab in these patients. If an infectious etiology is suspected, serology, PCR analysis or culture of the specific infectious agent are needed. In some situations, if a bacterial cause of the transverse myelitis cannot be ruled out initially, antibiotics with steroids may be started till reports of the AQP4 Ab status become available. It is worth noting that several days after zoster infection, some patients may develop seropositive NMOSD, reflecting some pathogenic overlap between these two disorders [35-36].

Conclusion

NMOSD is a common demyelinating disorder predominantly involving the optic nerves and spinal cord. This phenotype is shared by many other pathologies, including inflammatory, infectious, vascular and malignant causes. Early differentiation between these disorders is necessary to initiate treatment with immunosuppressives in NMOSD to prevent further relapses and accrual of disability.

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Chapter 5

Tapered Modular Femoral Components in Revision Total Hip Arthroplasty

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ABSTRACT

The burden of revision total hip arthroplasty continues to increase. These cases are often associated with compromised femoral bone stock making conventional prosthesis unsuitable. While this remains a technically challenging procedure, modular tapered femoral components are valuable tools in dealing with problems unique to femoral revision cases. Distal fixation bypasses proximal bone defects with predictable biologic fixation. Modularity allows more precise restoration of femoral offset and leg length, as well as correction of preexisting rotational deformities. However, the addition of a stem-proximal body junction increases the risk of failure due to fretting corrosion and fatigue fracture. Modular femoral components have added more options for revision total hip arthroplasty to improve stability and allow for osseointegration, but there remains a need for larger studies examining longer term outcomes greater than ten years for these components.

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Keywords: Total Hip Arthroplasty, Revision, Modularity, Femoral Fixation, Cementless

Introduction

The number of total hip arthroplasty (THA) revisions is projected to increase 137% by 2030 [1]. Surgeons handling revision of femoral components in THA must often deal with deficient bone stock while ensuring stable fixation and durable load-bearing capability of the revision component. In patients with substantial bone loss, cemented revision femoral components have shown aseptic loosening rates of 26% with older cementing techniques [2]. Newer cement techniques that maintain pressurization before and during stem insertion have shown to decrease rates of aseptic loosening by improving the shear strength [3]. However, adequate interdigitation of the cement to allow for stable fixation in the setting of increasing bone deficiencies and limited cancellous bone remains a concern. Extensively porous coated non-modular prostheses have shown excellent long-term results in femoral revision. While design and material properties of these components allow for axial and rotational stability, they offer less flexibility in version and offset, and higher failure rates in severely deficient femoral bone [4].

Modular femoral components with biologic fixation have provided an additional tool in the surgeon's armamentarium for femoral revision. While proximally porous-coated modular systems have performed well in the setting of minimal bone loss, they have shown poor survivorship with more severe bone loss [5,6]. Revision systems designed to gain reliable initial fixation with a high propensity for bony ingrowth in the diaphysis distal to bone deficiencies combats this issue. Incorporation of a separate proximal body with relative technical ease allows for restoration of femoral offset, version, and leg length independent of fixation distally. Despite these advantages, there has been concern about implant fracture at the modular junction as well as subsidence of the component.

Operative Indications and Planning

Indications for revision of a femoral component in THA are aseptic loosening, recurrent instability with malpositioned femoral component, breakage of component, infection, and osteolysis. There are also unique situations that necessitate femoral component for exposure purposes in order to achieve an adequate revision for acetabular pathology. Regardless of the indication, there is a high probability that changes to the proximal femur in the form of cortical hypertrophy, varus remodeling, and bone loss both from the index procedure and during the implant removal process will exist to some degree.

Preoperative planning, starting with a complete history and physical exam is essential with any THA revision. The surgeon should have a thorough understanding of the patient chief complaint and symptoms warranting the revision. A comprehensive past surgical history, particularly related to the spine and ipsilateral lower extremity in question, may reveal changes in alignment or risk factors for failure. Observation of the gait pattern, neurologic exam of the lower extremities, and assessment for previous scars or areas of skin compromise is critical. The implants being revised should be identified if at all possible, using operative reports if available. Implant manufacturers should be contacted to inquire whether there are extraction devices specific to the implant. It is useful to know the acetabular component manufacturer and design so that replacement liner sizes are available in case polyethylene exchange is needed or the acetabular component is found to be loose at the time of revision.

Serial plain radiographs are used to evaluate for osteolysis, stress shielding, cortical deficiency, and femoral deformity. X-rays of the entire femur are useful to evaluate for deformity, the existing bowing of the femur, and quality of bone stock distally. They are also valuable to evaluate if there is adequate bone for distal fixation. Consideration for cross sectional imaging may be given in select cases of concomitant osteolysis involving the acetabulum or a metal on metal articulation.

Assessment of radiographs over time are used to evaluate whether the stem is well-fixed or loose. Cemented components that have subsided or have a fracture either through the cement mantle or proximal femur are loose, while those that have a continuous radiolucent line around the bone-cement interface are likely loose [7]. Components with biologic fixation that have progressive subsidence, migration or divergent radiolucent lines are unstable [8]. Other indications of a loose component are a distal pedestal and proximal femoral hypertrophy. The Paprosky classification of

bony defects described below provides the surgeon with guidelines for stem selection based on the remaining quantity and quality of both metaphyseal and diaphyseal bone. The system has four categories based on the extent and location of femoral bone loss [9]. This classification system provides the basis for proper selection of the femoral revision component (Figure 1):

Type I
There is minimal loss of metaphyseal cancellous bone with an intact diaphysis. The femur resembles one seen in primary total hip arthroplasty [9] (Figure 1).

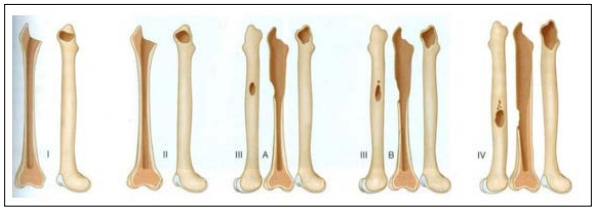

Figure 1: The Paprosky femoral classification of bony defects

Type II
In a type II deficiency, there is extensive loss of metaphyseal cancellous bone with an intact diaphysis. This is often seen after removal of a cemented component (Figure 1) [9].

Type IIIA
A femur with an IIIA defect has a severely damaged metaphysis that is unsupportive. There remains >4cm of intact diaphyseal bone at the isthmus observed after removal the present femoral component for distal fixation (Figure 1) [9].

Type IIIB
A type IIIB defect has extensive metadiaphyseal bone loss with <4cm of intact diaphyseal bone at the isthmus for fixation (Figure 1) [9].

Type IV
There is extensive metadiaphyseal damage with thin cortices and a widened canal causing the femoral isthmus to be nonsupportive (Figure 1) [9].

Surgical Technique
Pre-operative templating guided by a low AP pelvis and an orthogonal full-length femur films is essential for success. Flexible osteotomes, small diameter burrs, and explant equipment should be available. An extensive trochanteric osteotomy (ETO) may be used in cases where there is fixation of a native implant distally or a difficult cement mantle. This step may be essential if any varus remodeling has occurred if there is excessive anterior bowing of the femur to minimize the risk of perforation of the cortex [10]. If an ETO is used, a cerclage wire/cable may be placed one centimeter distally to the ETO to reduce risk of iatrogenic fracture during femur preparation and stem insertion.

All retained cement must be completely removed with a combination of curettes, chisels, burrs and ultrasonic bone cement removal instrument for osseous integration to occur. The diaphyseal femur is prepared with sequential reaming. Four centimeters of intact diaphyseal bone at the isthmus with a stem diameter of less than eighteen centimeters has been shown to be adequate when employing extensively porous cylindrical stems [11]. Tapered stems may only require 1.5-2.5 centimeters for acceptable biologic fixation [12]. When reaming, care should be taken to avoid perforation of the femur and the canal should be reamed line to line. Intra-operative imaging with the reamer in place may be useful to assess canal fit and if there is suspicion for perforation. The distal stem can be inserted after the canal has been reamed to the appropriate size. Curved or kinked stems can be used to address a femoral bow.

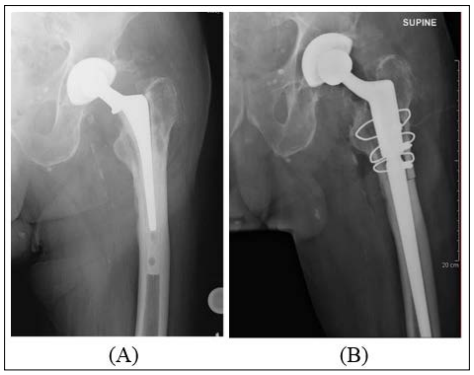

Figure 2: A, Pre-operative x-ray of a loose cemented stem. B, Post-operative x-ray after ETO and revision to a modular diaphyseal fixation stem.

Trialing can be performed after the distal stem is impacted. A separate proximal body piece with various lengths, neck length, and offsets can be placed onto the impacted distal stem. This gives the surgeon many options to restore leg length, femoral version and offset independent of distal fixation. After successful trialing, the body and femoral head are inserted. If an ETO was used, the fragment should be secured with two or three cerclage wires/cables to promote boney union and provide support at the modular junction (Figure 2).

Post-Operative Care
Patients are partial weight bearing with the aid of a walker or crutches for six to eight weeks. If an ETO was used, the patients are instructed to avoid active abduction for the same time frame. After six to eight weeks, the patient can begin progressive weight bearing and active abduction if there has not been displacement of the osteotomy or significant subsidence of the stem.

Outcomes
Amantullah et al. examined 92 patients treated with modular tapered femoral revision components who had mean clinical follow up time of 6.4 years (range 2-12 years). Four hips required removal of the distal femoral stem (three for infection and one for aseptic loosening). There were fourteen hips that were revised either for instability or failure of the acetabular component that did not involve removal of the distal stem. They reported no fractures of the femoral component [13]. Abdel et al. also examined patients treated with a modular taper fluted stem at mid-term to long-term intervals. The mean clinical follow up time for their 519 cases was 4.5 years (range 2 - 14 years). Similarly, the authors report ten-year survivorship to be 98%. Ten hips required revision of the femoral stem for infection or aseptic loosening. There were three revisions for instability. They observed subsidence in 12 cases [14]. Brown et al. also examined 135 cases which involved a modular taper stem that had mean clinical follow up time of 5.6 years (range 2 - 12 years). In the 58 cases with Paprosky IIIB or IV defects, Kaplan-Meier survivorship was 98% at 12 year and mean Harris hip score improved from a mean preoperative value of 34 to mean postoperative value of 74 [15]. Overall, survival outcomes related to this stem design continue to be > 95% at mid-term and long-term time points [16-21].

There are other retrospective series with smaller numbers, differing study designs, or shorter ranges follow up which highlight the utility modular taper stems. Richards et al. compared outcomes of tapered, fluted, modular stems and a cylindrical nonmodular stem [22-24]. While the tapered stem group was associated with significantly worse osseous defects, with 65% Paprosky IIIB or IV defects, this group achieved superior WOMAC and Oxford-12 scores when compared to the nonmodular group. The tapered group also had fewer fractures and more proximal osseous restoration. The stems had similar dislocation and deep infection rates. Studies using the Link MP design (Waldemar Link, Hamburg Germany) have reported an average of 2.1-2.7mm of subsidence over an average of 5-6 years of follow-up [16,26]. Other literature has shown a decrease in stress shielding proximally compared to extensively porous-coated implants with bony regeneration proximally in 73% of hips with only 6.2% subsidence [25]. In a series of 125 revisions, Ovesen et al. reported mean Harris hip score improvement from a mean preoperative value of 44 to mean postoperative value of 85. They did not revise any cases for subsidence and reported no increase in the overall rate of complications compared to the revision hip literature [17].

Nevertheless, higher rates of complications related to stem subsidence remain a concern. Patel et al. had a re-revision rate of 9.3% due to subsidence with an average subsidence in that group of 20 millimeters [29]. It was also found to be more likely in Paprosky type IIIB and IV femurs. Stems that subsided more than one cm were more likely to require revision. All but one of the subsided stems occurred early in their surgical experience with the implant (Restoration Modular and T3 Stryker Howmedica Osteonics, Mahwah, NJ). This demonstrates there is a learning curve when using these implants. Subsidence may be prevented by aggressive conical reaming to avoid undersizing of the implant.

Additionally, there is concern for complications around the modular junction. Efe et al. described four cases of stem fracture just distal to the stem-proximal body junction (Figure 3) [27]. All four stems remained firmly fixed distal to the stem fracture site. All cases showed evidence of proximal component loosening. Examination under electron microscope revealed fatigue fractures and analysis revealed evidence of gap corrosion. Busch et al. also identified 5 stem fractures and concluded that inadequate proximal bone support can increase the risk of stem fracture [28]. Risk factors for fracture were Paprosky type III or IV bone, BMI >30, smaller diameter stems, and use of extended trochanteric osteotomy. This is a rare complication with an incidence of 0.24% in Australian Registry data from 2003 [27].

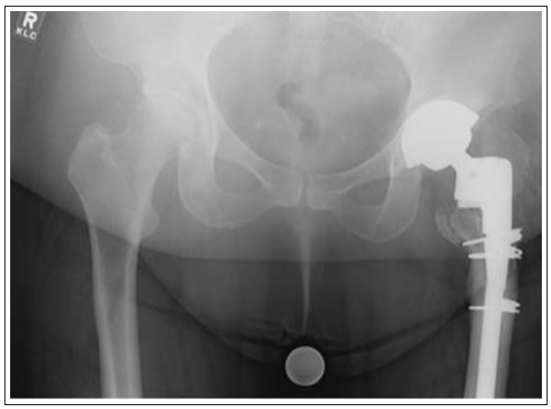

Figure 3: Pre-operative x-rays demonstrating a fracture of the femoral components distal to the modular junction

Conclusion

Management of femoral implant revision is based on the bony defects and bone quality. Modular tapered femoral components have added options for femoral revision in total hip arthroplasty. The components allow the surgeon to more closely restore proximal bone, offset, femoral version and leg length independent of distal fixation. There are concerns about stem fracture and component subsidence and limited long-term outcome data is available on implant survivorship. Based on short-term and mid-term data, it appears these components offer a viable option for the revision surgeon to address femoral deficiencies and achieve reliable osseointegration.

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