ISSN: 2634 - 1255

Journal of Gynecology Research Reviews & Reports

SCIENTIFIC Research and Community

Research Article

Open Access

Comparative Study of the Antioxidant Activity of the Plasma of Healthy Subjects and those with Uterine Myomas by Scavenging the ABTS Radical+

N'Negue Mezui-Mbeng MA[1]*, Makoyo Komba O[2]*, Bang Ntamack JA[2] and E Lendoye[1]

[1]Chemistry-Biochemistry Laboratory of the Faculty of Medicine. University of Health Sciences, Libreville-Gabon

[2]Gynecological service. Mother Child University Hospital Center Jeanne Ebori Foundation, Libreville-Gabon

ABSTRACT

Introduction: The oxidative stress and the deterioration of the antioxidant system demonstrated by the dosage of antioxidant enzymes and the by-products of lipid peroxidation are involved in the occurrence of uterine myomas.

Objectives: To measure the antioxidant activity of the plasma of patients with uterine myoma and that of the plasma of their age-matched controls by trapping the free radical ABTS+. The study aims to confirm by another assessment technique.

Results: The anti-free radical activities of the plasmas of patients with myomas and those of their respective controls vary from 1.3 to 6%. In addition, two cases were observed: either the anti-radical activity of the control subjects turned out to be slightly higher than that of the patients; or the anti-free radical activity of patients with uterine myomas was slightly stronger than those of their controls. In addition, our results showed that the anti-free radical activity of patients aged over 40 years is slightly higher than that of patients aged less than or equal to 40 years. Finally, the anti-free radical activity decreases with an increase in BMI and increases with the number of gestities and parity.

Conclusion: Assessment of the total antioxidant capacity of blood by trapping the ABTS+ radical can be used to assess, at a certain threshold, the oxidative stress involved in the development of uterine myoma. This method can also be used to assess the effect of age, BMI, pregnancy and parity on the occurrence of myomas.

***Corresponding author's**

Makoyo Komba Opheelia, Gynecological service. Mother Child University Hospital Center Jeanne Ebori Foundation, Libreville-Gabon. Tel No: +241 66 24 94 91. E-mail: mopheelia@yahoo.fr.

N Negue Mezui-Mbeng Marie-Andrée, Chemistry-Biochemistry Laboratory of the Faculty of Medicine, University of Health Sciences, Libreville-Gabon. Tel No: + 241 77 62 27 28, E-mail: negmarie@yahoo.fr.

Received: December 23, 2021; **Accepted:** December 31, 2021; **Published:** January 07, 2022

Keywords: Uterine Myoma, Plasma, Total Antioxidant Capacity, ABTS+

Introduction

Oxidative stress and alteration of the antioxidant system are believed to be involved in gynecological diseases including uterine myomas. Myomas are benign tumors very common in Black populations, with origin of which is unknown. The occurrence of a fibroid is said to be secondary to a genetic predisposition. Certain factors act on proliferation, in particular many growth factors such as TGFβ, EGF, βFGF and especially VEGF [1]. According to the literature, disturbances in antioxidant levels, and lipid peroxidation, are reported in patients with uterine myomas [2-3]. According to Nayki et al [4]. The oxidative-antioxidative status of the endometrium of patients diagnosed with uterine myomas varies compared to that of normal patients. Women with mild gynecological disorders such as myomas show impaired antioxidant enzyme activities. Caglayan et al. show that the erythrocyte activities of antioxidant enzymes (copper-zinc superoxide dismutase, catalase, glutathione peroxidase) are higher in women with myomas than in women without myomas; and that the plasma levels of malondialdehyde, by-products of lipid peroxidation were lower in patients with uterine myomas compared to controls [5].

A single nucleotide polymorphism of the paraoxonase 1 (PON1) gene, an enzyme playing a defensive role against oxidative stress, modifies the activity of the enzyme and leads to an increased risk of developing tumors, especially uterine myomas [6]. On the other hand, dietary supplementation with selenium known for its antioxidant properties has been shown to reduce the size of oviduct leiomyomas occurring spontaneously in Japanese quail [7].

Also if it is well established that oxidative stress and its consequences are involved in the development of uterine myoma in women of childbearing age, no study has however evaluated the overall antiradical status of the blood of patients diagnosed for uterine myomas. We therefore propose in this study to compare the total anti-free radical activity of the plasma of patients with myomas to that of the age-matched controls. The anti-radical activity will be measured by trapping the radical cation of 2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic] (ABTS•+) acid according to the method developed by Re et al. and optimized by N'negue et al. with Gallic acid as the standard antioxidant [8,9].

Materials and Methods

Sampling

Twenty blood samples were taken from women without any chronic pathology and from women with uterine myomas. ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]), gallic acid, potassium persulfate (K2S2O8) and hydrated sodium dihydrogen phosphate were purchased from Sigma-Adrilch (Saint -Quentin Fallavier, France).The water used was distilled by the equipment of the "Milli-Q Labo" laboratory (Millipore Japan, Tokyo, Japan). All these products are quality for analysis. The anti-radical activity was determined by UV spectrophotometry: V-200 spectrophotometer (BOECO, Germany). The optical density reading was taken at 734 nm, absorption wavelength.

Preparation of Blood Samples

The blood of 12 healthy subjects without any chronic pathology and that of 9 subjects with uterine myoma was drawn from the venous area in EDTA anticoagulant tubes in order to obtain plasma. The mean ages of women with myomas and healthy women were the same. All tubes were centrifuged at 3000 rpm for 5 minutes. Plasma samples were diluted 100X at the time of spectrophotometer reading.

Preparation of Gallic Acid Solutions, "Reference Antioxidant"

Gallic acid (3,4,5-trihydroxybenzoic acid) is an aromatic organic compound, used as a reference anti-radical compound. Ten working solutions, in decreasing concentrations, ranging from 0.5 to 5 µM, were prepared by diluting Gallic acid in distilled water.

Measurement of Anti-Radical Activity

The principle of the test for measuring the anti-radical activity by the ABTS method is based on the decrease in the absorbance at 734 nm of the radical cation ABTS • + (blue-green coloration) in the presence of a potentially anti- compound. Radical which reduces the cation radical. The reduction in the radical form of ABTS • + leads to a discoloration of the solution.The ABTS • + radical ion is obtained by reacting the ABTS molecule (7 mM) with potassium persulfate (2.45 mM), in distilled water for 16 hours at room temperature and under cover light.

Results

Validation of the ABTS Method with a Reference Antioxidant "Gallic Acid"

Anti-Free Radical Activity of Gallic Acid Depending on the Concentration

The percentage (%) of anti-radical activity increases linearly with the concentration of Gallic acid (Figure 1). The ABTS • + Radical disappears in the presence of the standard antioxidant. Indeed, an anti-radical activity of 14.34 ± 2.31% was recorded for a concentration of 0.5 µM of Gallic acid (0.37 µg.mL-1). This activity increases to 98.6 ± 1.98% for a total concentration of 4.5 µM. According to our results, the IC50 of gallic acid, which is the concentration necessary for the reduction by 50% of the anti-radical activity (or the disappearance of 50% of the radical form ABTS • +) is 2 µM (0.37 µg.mL-1).

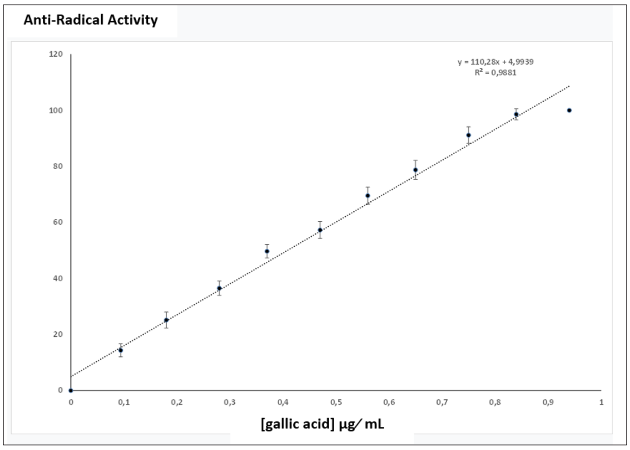

Figure 1: Anti-radical activity depending on the concentration of gallic acid after 6 minutes of incubation. The proportion ABTS•+ transformed into ABTS+ in the presence of Gallic acid is calculated from the change in absorbance at 734 nm measured by spectrophotometry. The equation on the right is: y = 110.28x + 4.9 (R2 = 0.98); n = 3.

General Characteristics of the Study Population

Patients with Uterine Myomas

No	Age	BMI	TAS/TAD	Pregnancies	Parity	Fruit/ vegetable	%AAR
1	24	17	99/65	2	0	1/day	28.32 ± 4,5
2	29	24.5	108/68	1	1	1/day	31.64 ± 1,7
3	36	31.25	90/75	5	5	1/day	32.77 ± 2,55
4	40	24.5	128/68	0	0	1/day	37.57 ± 0,29
5	44	39	102/69	7	3	1/day	40.18 ± 0,78
6	45	30.85	161/88	4	2	1/day	37.76 ± 1,92
7	45	29.07	105/73	1	1	1/day	26.642 ± 1,26
8	46	23.87	122/86	3	0	2-3/day	35.53 ± 1,76
9	47	34.40	180/84	4	1	1/day	31.16 ± 3,66
10	47	29	129/89	6	2	2-3/day	36.65 ± 1,35
11	47	25	132/85	7	3	1/day	37.47 ± 0,38
12	50	21	108/60	7	3	2/day	33.01 ± 1,49

Patientes Sans Myomes Utérins « Controls »

No	Age	BMI	TAS/TAD	Pregnancies	Parity	Fruit/ vegetable	%AAR
1	24	22.5	126/82	0	0	1/day	31.9 ± 0.01
2	29	30.45	119/85	2	1	1/day	29.71 ± 0.4
3	36	21.40	123/80	1	1	2-3/day	37.36 ± 1.4
4	40	2.5	110/60	1	0	1/day	34.7 ± 2.9
5	44	2.4	110/70	4	2	2-3/day	38.28 ± 0.9
6	45	23.90	101/66	4	3	2-3/day	32.55 ± 1.7
7	46	28.37	110/70	5	3	2-3/day	34.95 ± 1.4
8	47	26.50	1280/79	7	5	2-3/day	34.73 ± 0.5
9	50	29	135/87	7	4	1/day	34.28 ± 1.5

Notes: Based on the data listed in the tables above, there was no hypertension in the study population; 6 out of 12 people are overweight (BMI > 25) in patients with myomas and 5/9 in controls.

Comparison of the Anti-Radical Activity of Patients with Myomas and Their Controls without Myomas

The displayed results of the anti-radical activity of the plasma of the patients with uterine myomas and their age-matched controls are shown in Figure 2. According to these, the anti-radical activities of the patients with myomas and those of their respective controls vary by only 1.3 to 6%.

In addition, our results showed that depending on the situation, the anti-radical activity of the control subjects could be slightly higher than that of the patients (controls: 1 (24 years), 3 (36 years), 6 (45 years), 8 (47 years old), 9 (50 years old)). Or, conversely, the antiradical activity of patients with uterine myomas was slightly stronger than those of their controls (patients: 2 (29 years), 4 (40 years), 5 (44 years), 6 (45 years), 8 (46 years), 10 (47 years old), 11 (47 years old)).

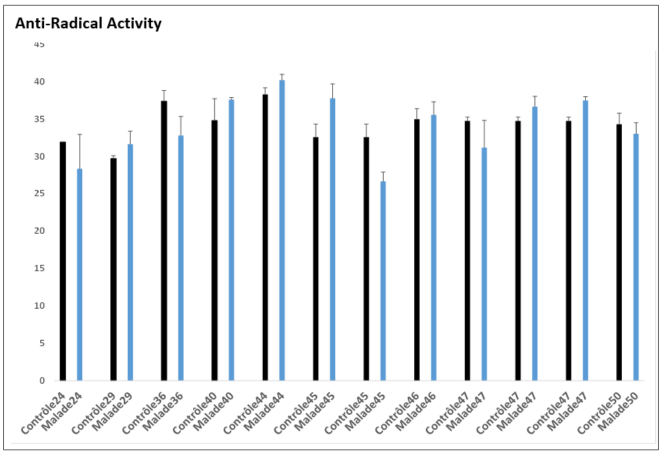

Figure 2: Anti-radical activity of various plasma samples (diluted to 1/00) from patients with myomas (Sick/Age) and their control subjects (control/Age) matched according to age. The proportion ABTS•+ transformed into ABTS+ in the presence of plasma samples is calculated from the change in absorbance at 734 nm measured by spectrophotometry; n = 2.

Effect of age on the anti-radical activity of patients with myomas

We divided the patients with myomas and their controls without myomas into three groups according to age, and we looked at the evolution of anti-radical activity in the 3 groups. Group 1 includes subjects aged less than 40 years; Group 2, those whose age was between 40 years inclusive and 45 years; Group 3 included subjects over 45 years. The results obtained are displayed in the Figure 4 below.

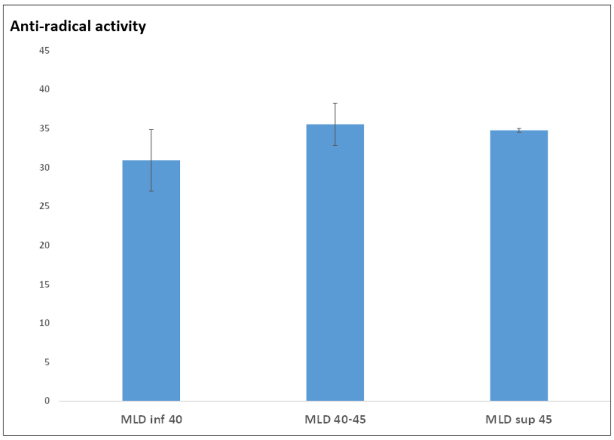

Figure 3: Anti-radical activity of different plasma samples (diluted to 1/00) from patients with myomas grouped by age group. The proportion ABTS•+ transformed into ABTS+ in the presence of plasma samples is calculated from the change in absorbance at 734 nm measured by spectrophotometry; n = 2. (MLD inf 40 = patients under 40 years; MLD 40-45 = patients aged between 40 years inclusive and 45 years inclusive; MLD over 45 = patients over 45 years).

Effect of BMI on Radical Activity in Subjects with Myomas

The evolution of the antiradical activity of the 2 patients aged 45 years with BMIs of 24 and 29 on the one hand, and that of the 3 patients aged 47 with BMIs of 25, 29 and 34 are presented in figure 4 According to the results, the anti-free radical activity decreases with increasing BMI. In fact, for the 2 patients aged 45, the anti-radical activity is 26.64 ± 1.26% for a BMI of 29 and 37.76 ± 1.92% for a BMI of 24.

The results obtained with the 3 patients aged 47 also showed a decrease in anti-radical activity with an increase in BMI. Indeed, for a BMI of 25, the anti-radical activity was 37.47 ± 0.48%; it was 36.65 ± 1.357% for a BMI of 29 and 31.16 ± 3.66% for a BMI of 34.

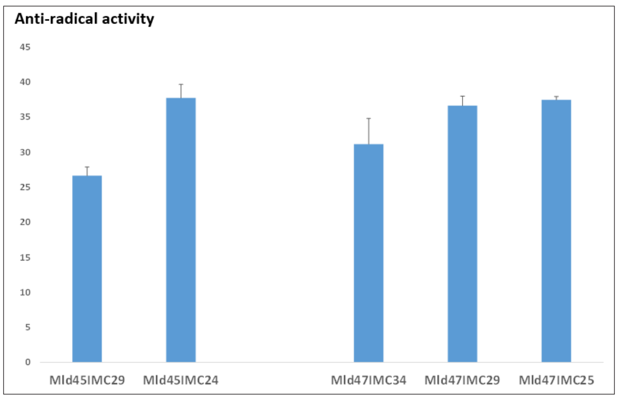

Figure 4: Anti-radical activity of various plasma samples (diluted to 1/00) from patients with myoma aged either 45 years with BMIs of 29 and 24, or 47 years with BMIs of 34, 29, and 25. The proportion ABTS•+ transformed into ABTS+ in the presence of plasma samples is calculated from the change in absorbance at 734 nm measured by spectrophotometry; n = 2 (Mld45IMC29 = 45-year-old patient with a BMI of 29; Mld45IMC24 = 45-year-old patient with a BMI of 24; Mld47IMC29 = 47-year-old patient with a BMI of 24; Mld47IMC29 = 47-year-old patient with a BMI of 29; Mld47IMC25 = patient aged 47 with a BMI of 25).

Effect of Pregnancy on the Anti-Radical Activity of Subjects with Myomas

The anti-radical activity was compared in two groups of patients with gestures from 0 to 3 for the first group and from 4 to 7 for the second. The results obtained are shown in Figure 5 below. According to these results, the average anti-free radical activity of the group of patients with 4 to 7 gestures was approximately 4% higher than that of subjects with myomas having a pregnancy between 0 and 3. In fact, the average anti-free radical activity is 31 , 94 ± 4.63% for patients with gestures between 0 and 3 and 35.57 ± 3.28% for those with gestures between 4 and 7.

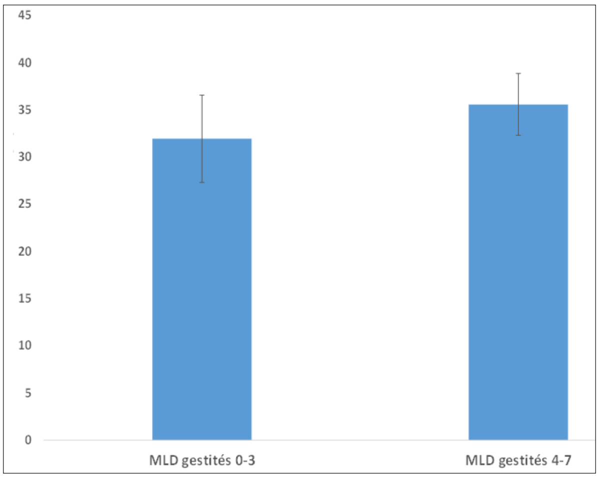

Figure 5: Anti-radical activity according to the gesture of the different plasma samples (diluted to 1/100th) from patients with myomas. The patients are grouped by number of gestures; from 0 to 3 gestations for group 1 and from 4 to 7 gestures for group 2. The proportion $ABTS^{\bullet+}$ transformed into $ABTS^{+}$ in the presence of plasma samples is calculated from the variation in absorbance at 734 nm measured by spectrophotometry; n = 2. (MDLgestity 0-3 = patients with a pregnancy count ranging from 0-3; MDL 4-7 = patients with a gestational count ranging from 4-7).

Effect of Parity on the Anti-Radical Activity of Subjects with Myomas

The anti-radical activity was compared in patients with parities of 0 to 2 for the first group and of 3 to 5 for the second group. The results obtained are presented in Figure 6. According to these results, the average anti-radical activity of the group of patients with parities of 3 to 5 was approximately 3% higher than that of women with myoma with parities of 0 to 2. In fact, the average anti-free radical activity is 35.96 ± 3.12% for patients with parities from 3 to 5 and 33.16 ± 3.04% for those whose parities are from 0 to 2.

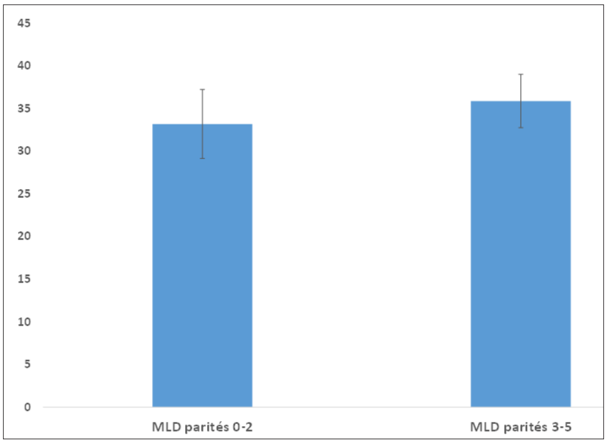

Figure 6: Anti-radical activity as a function of the parity of the different plasma samples (diluted to 1/00th) from patients with myomas. The patients are grouped together in number of parity. From 0 to 2 parities for group 1 and from 3 to 5 parities for group 2. The proportion ABTS•+ transformed into ABTS+ in the presence of plasma samples is calculated from the variation in absorbance at 734 nm measured by spectrophotometry ; n = 2. (MDLparities 0-2 = patients with a parity number ranging from 0-2; MDLparity 3-5 = patients with a parity number ranging from 3-5).

Discussion

Our study compared the antioxidant activity of plasma (diluted to 1/100th) of patients with uterine myomas to that of age-matched controls. This is to confirm or not the involvement of oxidative stress in the etiology of uterine myoma. The anti-radical activity was measured by trapping the free radical ABTS+ According to the method of Re et al. optimized by N'negue et al. with gallic acid as the standard antioxidant [8,9]. In this method, the concentration of the radical cation ABTS•+, blue-green in color absorbing at 734 nm, decreases in the presence of an antioxidant compound capable of trapping said radical and reducing it to a non-radical ABTS+ molecule.

This will result in a decrease in absorbance at 734 nm and a discoloration of the solution. According to Fischer et al., this test can be applied routinely to predict the total antioxidant capacity of the blood [10]. The results of the antioxidant activity of gallic acid (a synthetic strongly antioxidant molecule) validate the chosen method (Figure 1). The IC50 value of gallic acid deduced from our results was 2 μM. This value is equivalent to that obtained by Sadat et al. and N'negue et al., which worked under the same conditions [9, 11, 12].

The results of the anti-radical activity of the plasma of patients with uterine myoma and of their age-matched controls (Figure 2) showed a variation of 1.3 to 6% depending on the couple. In addition, our results showed that depending on the situation, the anti-radical activity could be slightly higher in the control subjects compared to the patients or vice versa in the patients compared to the controls. Nayki et al. observed either a decrease or an insignificant increase in the activity of the antioxidant enzymes "superoxide dismutase, catalase, glutathione reductase" in the endometrium of patients with uterine myoma, compared to normal women [4]. Their results appear to be equivalent to ours, although the technique for assessing antioxidant activity was not the same.

The decrease or increase in the anti-radical activity of the plasma of subjects with myomas may reflect the existence of mild oxidative stress in the blood of the patients. Indeed, the decrease in the anti-free radical activity of the plasma of myoma patients can be explained by a prior internal reaction between the non-enzymatic anti-free radical molecules in the blood (total phenolic compounds, flavonoids, uric acid, ascorbic acid, etc. alpha-tocopherol, beta-carotene, reduced glutathione (GSH), bilirubin, lipid peroxidation products, etc.) and plasma free radicals resulting from oxidative stress. This decreases the level of anti-radical molecules in plasma capable of trapping the free radical ABTS+, with a decrease in the anti-radical activity measured. Likewise, the increase in anti-free radical activity in the plasma of patients with myomas can be explained by an increase in the synthesis of antioxidant molecules in order to combat oxidative stress.

Moreover, Caglayan et al. showed that the erythrocyte activities of antioxidant enzymes (copper-zinc superoxide dismutase, catalase, glutathione peroxidase) are higher in women with myomas than in women without myomas [5]. In addition, ccording to Fletcher et al., fibroid cells exhibit significantly elevated levels of antioxidant enzymes (superoxide dismutase and catalase) compared to normal cells, indicating the existence of oxidative stress [13].

The effect of age on the anti-radical activity of women with uterine myomas (Figure 3) showed that the anti-radical activity of patients over 40 years of age was slightly higher than that of patients of less than or equal ages at 40. If oxidative stress results in increased oxidative activity, and oxidative stress is involved in the etiology of uterine myomas [13]. We can therefore deduce that in the uterus of women over 40 years of age, there would be more free radicals and oxidation reactions causing a slight oxidative stress. Moreover, according to the literature, age is one of the risk factors for the development of uterine fibroids [14]. The presence of fibroids is definitely very important from the age of 40. Uterine fibromyomas are present in 25-30% of women over 35, 40% of women over 40 and 50% of women over 50 [14].

The effect of BMI on the anti-radical activity of patients with myomas (Figure 4) showed a decrease in the anti-free radical activity of the plasma of subjects with myomas with increasing BMI and therefore with overweight. According to the literature, being overweight, due to physical inactivity, is a risk factor for the development of uterine fibroids [14]. The decrease in anti-radical activity in patients with myomas and overweight compared to those with myomas and normal build confirms the effect of overweight as a risk factor for the development of uterine myomas. Indeed, obesity can result in the appearance of chronic diseases (cardiovascular diseases, diabetes, etc.) in which oxidative damage with oxidative stress is present, oxidative stress linked to overweight decreases. Anti-radical activity in overweight patients [15-16].

The effect of pregnancy and parity on the anti-radical activity of patients with myomas (Figures 5 and 6) showed that the average anti-radical activity of the group of patients with a gestity between 4 and 7 was 4% higher approximately than that of subjects with myomas having a gestity between 0 and 3. Groups whose parity was 3 to 5 had an anti-free radical activity greater by approximately 3% than those whose parity was between 0 and 2. Parity would have protective effects against the appearance of oxidative stress and therefore by extrapolation, against the appearance of uterine myomas. According to the literature, nulliparity is one of the risk factors for the development of fibroids. Our results more or less confirm this result. This is because high anti-free radical activity may mean that there are fewer free radicals or reactive oxygen species (ROS) in the plasma of women with myomas with high pregnancy and parity rates.

Conclusion

Determination of the total antioxidant capacity of blood by scavenging the ABTS+ radical can be used to demonstrate oxidation reactions and oxidative stress in the plasma of patients with uterine myomas. It also makes it possible to assess the effect of age, BMI, pregnancy and parity factors on the occurrence of myomas. The results obtained by the ABTS method supplement those methods for evaluating oxidative stress by assaying the concentrations of antioxidant enzymes and secondary metabolites resulting from the reactions of oxidations of biomolecules (lipids, proteins, carbohydrates, DNA). Also, since oxidative stress plays a role in the etiology of uterine myomas, a diet rich in antioxidant compounds such as fruits and vegetables may play a preventative role in the development of uterine myoma.

References

1. Maitre S.C, Hugon (2009) J Physiopathologie des fibromes. Médecine de la Reproduction 11: 123-127.
2. Chiou JF, Hu ML (1999) Elevated lipid peroxidation and disturbed antioxidant enzyme activities in plasma and erythrocytes of patients with uterine cervicitis and myoma. ClinBiochem 32: 189-192.
3. AwukuAsare G, Akuffo G, Doku D, Asiedu B, Santa S (2016) Dynamics of urinary oxidative stress biomarkers : 8-hydroxy-2'-deoxyguanosine and 8-isoprostane in uterine leiomyomas. J Midlife Health 7 : 8-14.
4. Nayki C, NaykiU, GunayM, KulhanM, ÇankayaM, et al. (2017) Oxidative and antioxidative status in the endometrium of patients with benign gynecological disordersJ GynecolObstet Hum ReprodNutr 46 : 243-247.
5. Caglayan A, Katlan DC, Tuncer ZS, Yuce K, Sayal HB, et al. (2018) Assessmentof oxidant-antioxidant status alterations with tumor biomarkers and reproductive system hormones in uterine MYOMAS. Eur J ObstetGynecolReprodBiol 229: 1-7.
6. Shahbazi S, Zarei SZ, Torfeh M, Fatahi N (2020) Q192R variant in paraoxonase 1 gene confers susceptibility to leiomyoma. J Cancer Res Ther 16: 884-887.
7. Tuzcu M, Sahin N, OzercanI, SerenS, SahinK, et al. (2010) The effets of selenium supplementation on the spontaneously occurring fibroid tumors of oviduct, 8-hydroxy-2'-deoxyguanosine levels, and heat shock protein 70 response in Japanese quail. Nutr Cancer 62: 495-500.
8. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, et al. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical BioMed 26: 1231-1237.
9. N'Negueép, Mezui-Mbeng, Ella Ndong JG, NguemaEdzang RW, Lendoye E, etal., (2020) Kinetics of the study of the antioxidant activity of an aqueous extract of dried calyxes of Hibiscussabdariffa by trapping the radical ion ABTS IJAR 6: 361-367.
10. Fischer MA, Gransier TJ, Beckers LM, Bekers O, Bast A, et al., (2005) Determination of the antioxidant capacity in blood. Clin Chem Lab Med 43: 735-740.
11. Sadat L, Cakir-Kiefer C, Marie-Andrée, N'Negue MA, Gaillard JL (2011) Isolation and identification of antioxidative peptides from bovine α-lactalbumin. Int Dairy J 21: 214-221.
12. N'Negueép, Mezui-Mbeng MA, Lendoye E, Nguema Edzang RW, Ella Ndong JG, et al., (2021) Evaluation of the anti-radical activity of methanolicand aqueous extracts of stem, stem bark and leaves of Waltheriaindica by scavenging the free radical cation ABTS, Plants 9 : 1320.
13. Fletcher NM, Saed MG, Abu-Soud HM, Al-Hendy A, Diamond MP,et al., (2013) Uterine fibroids are characterized by an impaired antioxidant cellular system: potential role of hypoxia in the pathophysiology of uterine fibroids. J Assist Reprod Genet 30: 969- 974.
14. Commandeur AE, StyerAK (2015) Teixeira J.M.Indices épidémiologiques et génétiques des mécanismes moléculaires impliqués dans le développement et la croissance des léiomyomes utérins.Hum Reprod Update 21: 593-615.
15. Choi S, Liu X, Pan Z (2018) Zinc deficiency and cellular oxidative stress: prognostic implications in cardiovascular diseases. ActaPharmacol Sin 39: 1120-1132.
16. Luc K, Schramm-Luc A, Guzik TJ, Mikolajczyk TP (2019) Oxidative stress and inflammatory markers in prediabetes and diabetes. J PhysiolPharmacol https://pubmed.ncbi.nlm.nih.gov/32084643/.