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In Vitro Evaluation of Remineralization Potential: Whey Extract, Fluoride Varnish, and Fluoride Mouthwash on Bleached Enamel

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ABSTRACT

Background: Extrinsic discoloration is treated with bleaching which increases enamel porosity and roughness. Remineralizing materials is applied to avoid the bleaching side effects. The current study aimed to evaluate time dependent safety of bleaching materials on enamel surfaces roughness, and to assess the remineralizing potential of whey extract as a natural product and to compare it with the commercially available fluoride varnish and fluoride mouthwash after maximum bleaching time (30 min.).

Methods: Extracted human incisor teeth without cracks or any enamel defects were used in this study. Teeth were cleaned and decoronated at the level of the cementoenamel junction. A nail varnish was used to cover the entire surface of the crowns of each tooth, leaving a window measuring 4 mm × 4mm. Bleaching material was applied to the enamel of all teeth specimens. The Application of bleaching material was repeated 2 more times, each time for 10 minutes, which amounted in total to 30 min. The specimens in group 1 were soaked in artificial saliva, the specimens in group 2 were soaked in whey extract, the specimens in group 3 were treated with fluoride varnish and group 4 specimens were soaked in fluoride mouthwash.

Results: The mean value of the surface roughness of all the teeth specimens was increased significantly after bleaching for 10 minutes, 20 minutes and 30 minutes ($p=0.000$) when compared with baseline mean value. As well, there was significant direct correlation between application time of bleaching material and mean value of surface roughness ($r= 0.94$). There were no significant differences ($p>0.05$) between the specimens of the 4 groups when the mean value of surface roughness compared at baseline and after bleaching. But, after treatment, there was significant difference ($p\leq0.05$) between the 4 groups, with the best result obtained from whey extract and fluoride varnish.

Conclusions: The enamel surface roughness is significantly and directly correlated with prolonged application time of bleaching material. Remineralizing materials; whey extract, fluoride varnish and fluoride mouth wash had significantly decreased enamel surface roughness caused by bleaching. Whey extract has statistically the same effect as fluoride varnish on decreasing the surface roughness of bleached enamel surface.

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Introduction

Tooth discoloration can be categorized into intrinsic and extrinsic types. Extrinsic tooth discoloration, the more prevalent form, results from external factors affecting the enamel surface. This discoloration is often linked to the consumption of various food substances, colored beverages, or tobacco [1].

Bleaching is a common approach for treating extrinsic discoloration. This process involves the oxidation reaction of a bleaching agent with the tooth enamel structure. Hydrogen peroxide (H_2O_2) stands out as one of the widely used bleaching materials, available in different concentrations. Bleaching can be administered professionally or through self-application, each having varying contact times with the tooth enamel [2].

The use of bleaching materials induces changes in the enamel structure, often resulting in tooth sensitivity. The effectiveness of bleaching, alterations in the enamel surface, and associated side effects are primarily contingent on the diffusion capacity of the bleaching agent. This capacity is influenced by both the concentration of the bleaching material and the duration of its contact with the enamel [3]. Throughout the bleaching process, free radicals are generated and react non-selectively with the organic structures of the enamel [4].

Heshmat et al.'s research uncovered noteworthy changes in enamel microstructure following bleaching, including demineralization, degradation, heightened enamel microporosity, reduced microhardness, and increased surface roughness [5]. Surface roughness, characterized by irregularities resulting from the demineralization process, poses a risk of bacterial attachment, maturation, and stain absorption on the enamel. Post-bleaching, there was a significant increase in plaque accumulation by the fifth day compared to teeth without bleaching procedures. This

heightened plaque accumulation can potentially serve as a catalyst for the development of caries and periodontal diseases [6].

Mitigating the side effects of bleaching involves the application of remineralizing materials to replenish lost tooth minerals. Remineralizing agents typically consist of fluoride, calcium, and phosphate [7].

Fluoride stands out as the gold standard for tooth enamel remineralization. Its role includes inhibiting demineralization and promoting remineralization by facilitating the formation of fluorapatite crystals, which exhibit greater resistance to acid attacks compared to hydroxyapatite crystals [8]. Additionally, fluoride hinders the acid activity produced by carious bacteria [9].

While fluoride remains a valuable material in tooth remineralization, its use is constrained by the risk of fluorosis when applied in high concentrations. To address this concern, researchers are exploring natural alternatives that offer beneficial remineralization effects without the potential hazards associated with fluoride [10].

Whey extract, derived from dairy products and rich in casein phosphopeptide amorphous calcium phosphate (CPP-ACP), emerges as a noteworthy natural remineralizing agent [11, 12]. In a study by Rezvani et al., the impact of whey extract on enamel remineralization was assessed and compared with artificial saliva. The findings revealed a significantly higher remineralization effect in the whey extract group [13].

The current study aimed to evaluate the time-dependent safety of bleaching materials on enamel surface roughness, assess the remineralizing potential of whey extract as a natural product and compare it with the commercially available fluoride varnish and fluoride mouthwash after maximum bleaching time. Two null hypotheses were established, the first one is that different application times have no effect on enamel surface roughness and the second is that the application of the different remineralizing products on bleached enamel will not produce any variation in its surface roughness.

Materials and Methods

This study was an in vitro clinical trial. It was approved by the Ethics Committee of Pharos University, Faculty of Dentistry, under protocol no: (02-2023-8-29-3-127). G*Power 3.1 software was used to determine the minimum sample size, by 95% statistical significance, 0.80 test power, 0.60 effect size, and 4 experimental groups [13]. the minimum calculated required sample size was 77 rounded to 80 specimens (n =20/group). Freshly extracted human incisor teeth without cracks or any enamel defects were used in this study.

Preparation of the Specimens

The chosen extracted teeth underwent thorough cleaning to eliminate debris and organic materials. Subsequently, the roots were removed at the cementoenamel junction level. To isolate a specific area for examination, a nail varnish was applied to cover the entire crown surface of each tooth, creating a window measuring 4 mm × 4 mm. These treated tooth specimens were then stored in distilled water at room temperature until ready for use.

Whey Extract Preparation

Whey extract was produced using frozen plain yogurt of a thick consistency. This process involved centrifugation at 4000 rpm at a temperature of 25°C for 10 minutes, repeated over three cycles [14]. The preparation of this whey extract took place in the Biochemistry Department of the Faculty of Medicine at Alexandria University.

Bleaching

The bleaching process involved the application of 37% hydrogen peroxide (H_2O_2) to the enamel of all tooth specimens, utilizing a syringe for precision. This bleaching material was left on the enamel for 10 minutes before being wiped off with cotton. Following this, the specimens were rinsed with water and dried. This procedure was repeated two additional times, with each application lasting 10 minutes, culminating in a total treatment duration of 30 minutes. The roughness of the enamel surface was assessed at baseline and after each 10-minute interval of bleaching material application.

Grouping and Treatment (Figure 1):

Teeth specimens were divided randomly into 4 groups each group was 20 specimens:
Group 1: Control group (no remineralizing material).
Group 2: Whey extract (daily).
Group 3: 5% Sodium Fluoride (NaF) varnish (once).
Group 4: 0.05% Sodium Fluoride (NaF) mouthwash (daily).
The specimens in group 1 underwent a 14-day immersion in artificial saliva. For group 2, specimens were soaked in whey extract for one minute every 12 hours. group 3 specimens received a single application of fluoride varnish at the beginning of the study. In group 4, specimens were immersed in fluoride mouthwash for one minute every 12 hours.

Throughout the study, all specimens from each group were consistently soaked in artificial saliva, consisting of $CaCl_2$_$2H_2O$ (0.7 mmol/L), MgCl2 (0.2 mmol/L), KH_2PO_4 (4.0 mmol/L), HEPES buffer (as an acid form of 20.0 mmol/L), and KCl (30.0 mmol/L). The artificial saliva was replenished every 12 hours [15].

An enamel surface roughness test was performed for all teeth specimens at three different points. The surface roughness was quantitatively tested using a profilometer (Surfcorder se-1700, Japan) with an accuracy of 0.004 μm, and a precision level of 0.003 μm. The roughness test was conducted at the Dental Biomaterial Department, Faculty of Dentistry, Alexandria University. Surface roughness was examined at baseline, after bleaching at 10, 20 and 30 min and the end of the study after 14 days.

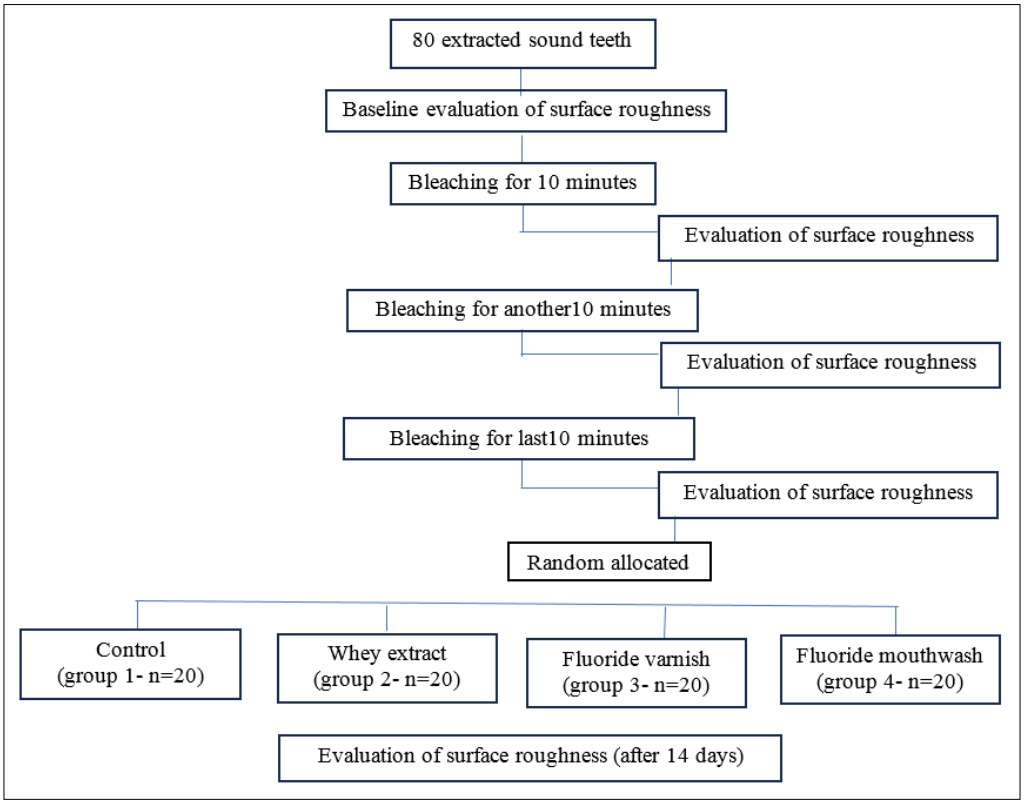

Figure 1: Grouping and Treatment

Statistical Analysis

The data of the mean value of enamel surface roughness was used for statistical analysis. The data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) version 25. The normality test was done using the Saphiro-Wilk method, while the homogeneity test uses the Levene-test method. Repeated ANOVA was used to analyze the mean value of surface roughness at different follow-up times. One Way ANOVA statistic test was used to analyze the mean value of surface roughness between groups, and then Least Significance Difference (LSD) was tested with 95% confidence level and $\alpha = 0.05$ to know whether there was any significant difference between pairs.

Results

The Saphiro-Wilk test proved that the data were normally distributed ($p > 0.05$) while the Levene test showed that the data were homogeneous ($p > 0.05$).

The results in Table 1 and Figure 2, found that the mean value of the surface roughness of all the teeth specimens at baseline was 0.134±0.027 then increased significantly after bleaching for 10 minutes (0.238±0.012), 20 minutes (0.346±0.030) and 30 minutes (0.448±0.019) the significance difference was found between each pair of different bleaching time ($p=0.000$). Also, there was a significant direct correlation ($p=0.000$) between bleaching application time and the mean value of surface roughness ($r= 0.94$).

Table 1: Relation Between Bleaching Time and Mean Values of Surface Roughness

Bleaching time	N	Mean	SD	Std. Error	95% Confidence Interval		F	P value	Correlation	P value
					Lower Bound	Upper Bound				
baseline	80	0.134[a]	0.027	0.003	0.128	0.140	**77112.2**	**0.000***	**0.941**	**0.000***
Bleaching (10 min)	80	0.238[b]	0.012	0.001	0.235	0.241				
Bleaching (20 min)	80	0.346[c]	0.030	0.003	0.339	0.353				
Bleaching (30 min)	80	0.448[d]	0.019	0.002	0.444	0.452				

*:Statistically significant difference ≤ 0.05
Different superscripts letters denote significant pairwise comparisons between different groups.

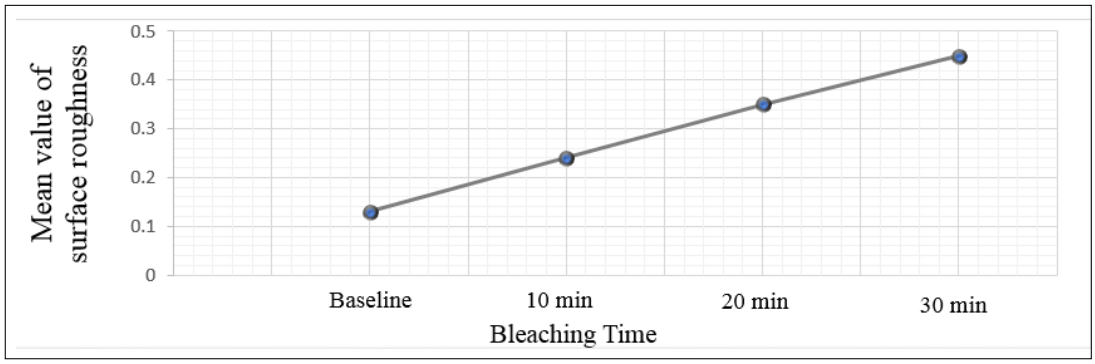

Figure 2: Relation Between Bleaching Time and Mean Values of Surface Roughness

Table 2 shows the comparison between the four groups regarding the mean values of surface roughness at baseline, after maximum bleaching time (30 min) and after treatment. At baseline, there were no significant differences (p=0.457) between group 1 (0.13±0.02), group 2 (0.14±0.01), group 3 (0.13±0.02) and group 4 (0.12±0.04). Furthermore, there was no significant differences (p=0.686) between the four groups after bleaching for 30 minutes with mean values of surface roughness (0.45±0.02, 0.45±0.02, 0.44±0.02 and 0.44±0.02 respectively). However, after treatment, there was significant difference between the four groups. Group 2 and 3 had significantly the least mean value of surface roughness ((0.16±0.05 and 0.13±0.02 respectively) followed by group 4 (0.19±0.04) and finally group 1 (0.42±0.01).

Table 2: Comparison Between 4 Groups Regarding Mean Values of Surface Roughness at Baseline, after Maximum Bleaching Time (30 min) and after Treatment

		N	Mean	SD	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
Baseline	Group 1 (control)	20	0.13	0.02	0.004	0.12	0.14
	Group 2 (whey extract)	20	0.14	0.01	0.003	0.13	0.15
	Group 3 fluoride varnish	20	0.13	0.02	0.004	0.12	0.15
	Group 4 (fluoride mouthwash)	20	0.12	0.04	0.010	0.12	0.14
	F	0.877					
	P value	0.457					
Bleaching (30 min)	Group 1 (control)	20	0.45	0.02	0.004	0.44	0.45
	Group 2 (whey extract)	20	0.45	0.02	0.004	0.44	0.45
	Group 3 fluoride varnish	20	0.44	0.02	0.005	0.43	0.45
	Group 4 (fluoride mouthwash)	20	0.44	0.02	0.004	0.44	0.46
	F	0.497					
	P value	0.686					
After treatment	Group 1 (control)	20	0.42a	0.01	0.003	0.41	0.42
	Group 2 (whey extract)	20	0.16b	0.05	0.013	0.12	0.18
	Group 3 fluoride varnish	20	0.13b	0.02	0.004	0.12	0.14
	Group 4 (fluoride mouthwash)	20	0.19c	0.04	0.009	0.17	0.21
	F	**246.277**					
	P value	**0.000***					

*:Statistically significant difference ≤ 0.05
Different superscripts letters denote significant pairwise comparison between different groups

When the comparison was made among all the study groups between baseline, after bleaching for 30 minutes and after treatment at the end of the study (Table 3), it was found that the mean value of surface roughness of the specimens of group 1 after treatment (0.42±0.01) was significantly higher than the value obtained at the baseline (0.13±0.02) and significantly lower than the value obtained after bleaching (0.45±0.02) (p=0.000). Regarding group 2 and 3 there were no significant differences (p>0.05) between the mean values of surface roughness after treatment (0.16, 0.05 and 0.13±0.02 respectively) when compared with the mean values at baseline (0.14±0.01and 0.13±0.02 respectively), while, there was high significant differences (p=0.000) with the mean values after bleaching (0.45±0.02 and 0.44±0.02 respectively). Although, in group 4, the mean value of surface roughness after treatment (0.19±0.04) decreased significantly than the mean value obtained after bleaching (0.44±0.02), as well, there was statistically significant difference between baseline (0.12±0.04) and after treatment (0.19±0.04) (p=0.000).

Table 3: Comparison Between Baseline, After Bleaching and After Treatment Mean Values of Surface Roughness among the 4 Groups

		N	Mean	SD	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
Group 1 (control)	Baseline	20	0.13a	0.02	0.004	0.12	0.14
	Bleaching (30 min)	20	0.45b	0.02	0.004	0.44	0.45
	After treatment	20	0.42c	0.01	0.003	0.41	0.42
	F	**22838.995**					
	P value	**0.000***					
Group 2 (whey extract)	Baseline	20	0.14a	0.01	0.003	0.13	0.15
	Bleaching (30 min)	20	0.45b	0.02	0.004	0.44	0.45
	After treatment	20	0.16a	0.05	0.013	0.12	0.18
	F	1377.321					
	P value	**0.000***					
Group 3 (fluoride varnish)	Baseline	20	0.13a	0.02	0.004	0.12	0.15
	Bleaching (30 min)	20	0.44b	0.02	0.005	0.43	0.45
	After treatment	20	0.13a	0.02	0.004	0.12	0.14
	F	1748.140					
	P value	0.000*					
Group 4 (fluoride mouthwash)	Baseline	20	0.12a	0.04	0.010	0.12	0.14
	Bleaching (30 min)	20	0.44b	0.02	0.004	0.44	0.46
	After treatment	20	0.19c	0.04	0.009	0.17	0.21
	F	**701.548**					
	P value	**0.000***					

*:Statistically significant difference ≤ 0.05
Different superscripts letters denote significant pairwise comparison between different groups

Discussion

The growing interest in aesthetic dentistry has led to an increased use of tooth bleaching, recognized as one of the most effective and conservative treatments for tooth discoloration [16]. High concentrations of hydrogen peroxide (H_2O_2), ranging from 20-45%, are commonly employed in dental clinics for bleaching. However, due to its low molecular weight, H_2O_2 can rapidly diffuse into the tooth structure, even reaching the pulp chamber. This penetration often results in tooth sensitivity, attributed to the release of inflammatory mediators [17,18]. Additionally, H_2O_2 can induce microscopic alterations in the tooth, such as increased porosity and surface roughness [19]. These adverse effects are largely dependent on the concentration of peroxide used and the duration of its application [20]. To counteract these effects, the application of remineralizing products post-whitening can help repair the morphological defects and mineral loss caused by the whitening agents [21].

The present study was conducted to evaluate the effect of bleaching time on enamel surface roughness and to compare the remineralization potential of whey extract which is a diary product containing CPP-ACP, fluoride varnish and fluoride mouthwash in the presence of control group, from the obtained results the null hypothesis of the current study was rejected.

The current study revealed a notable increase in enamel surface roughness, which directly correlated with the extended duration of bleaching material application. In 2022, Yang et al. investigated the effects of 35% H_2O_2 bleaching, with treatment times varying from 5 to 20 minutes. They evaluated both tooth color and enamel surface roughness. Their findings indicated that a 5-minute bleaching treatment achieved about 60% of the maximum bleaching effect, with the effectiveness at 10 minutes being nearly equivalent to that at 15 minutes. However, they observed that surface roughness increased with longer bleaching application times. Consequently, Yang et al. concluded that a bleaching duration of 5-10 minutes was optimal for achieving desirable tooth whitening effects while minimizing impact on tooth enamel [22].

Similarly, in 2023, Altınışık et al. conducted a study to assess the impact of in-office bleaching on the roughness and color stability of enamel surfaces, employing two sessions of H_2O_2 application. The assessments were made at baseline, after the first session, and then following the second bleaching session. Their findings suggested that a short exposure time is preferable for bleaching, as there was no significant difference in tooth color between the first and second sessions, yet surface roughness increased considerably after the second session [23].

Changes in enamel surface roughness during bleaching treatments are more closely associated with unnecessarily prolonged exposure times [24]. Reducing the duration of bleaching sessions can help in achieving effective tooth whitening while mitigating the adverse effects on enamel roughness.

Various remineralizing products have been explored to mitigate the adverse effects of bleaching procedures. Fluoride, being the initial and widely used compound in post-bleaching treatment, gained popularity for its capacity to facilitate mineral deposition on enamel surfaces [25]. The current study's findings revealed

significant differences in mean surface roughness among the four groups after treatment. The most favorable outcome was observed following the application of whey extract and fluoride varnish, followed by fluoride mouthwash, and lastly, the control group where specimens were maintained in artificial saliva only. Furthermore, when comparing baseline, post-bleaching (30 min), and post-treatment mean values of enamel surface roughness, the mean values after whey extract and fluoride varnish treatment returned to baseline levels, significantly lower than the values obtained after bleaching. However, the mean surface roughness values after mouthwash treatment exhibited a significant decrease compared to those after bleaching, although they did not return to baseline levels. The same pattern was observed for the control group.

In a 2018 in vitro study, the impact of whey extract and CPP-ACP on enamel surface roughness after bleaching was investigated. The findings demonstrated a significant increase in enamel surface remineralization after the application of whey extract over a 15-day period [25]. Another study in 2021 aimed to assess the remineralizing potential of whey extract, comparing it with commercially available minimal intervention (MI) varnish containing fluoride and Xylitol mouthwash. The results revealed that the maximum remineralization potential was observed in the MI varnish group, followed by the whey extract and mouthwash group, aligning with the outcomes of the current study. The effectiveness of MI varnish was attributed to the presence of fluoride [14].

A study focused on examining the effectiveness of 5% sodium fluoride varnish for remineralization after bleaching concluded that sodium fluoride varnish contributed to the formation fluorapatite [26]. Topical fluoride application post-bleaching has been shown to significantly reduce mineral loss, restore microhardness, and enhance enamel resistance to demineralization [27].

In an investigation comparing different remineralizing agents on enamel surface roughness, fluoride varnish and CPP-ACP were found to have similar effects in decreasing enamel surface roughness [28]. Marinho et al. also compared the effectiveness of fluoride varnish and mouthwash for remineralization, favoring varnish over mouthwash, potentially due to its ability to maintain prolonged contact time with the tooth surface. Insoluble globules of calcium fluoride formed on the tooth surface after topical fluoride application act as a reservoir for fluoride over an extended period [29, 30].

While acknowledging the comparable effects of whey extract as a natural product on enamel remineralization, attributed to its high content of calcium, phosphate ions, and casein in dairy products, it is suggested that whey extract may adsorb milk proteins, generating significant amounts of phosphorus and calcium. This, in turn, helps prevent demineralization [31].

Conclusions

Despite the limitations of this in vitro study, it was deduced that enamel surface roughness is noticeably and directly linked to the extended application time of bleaching material. Remineralizing agents; whey extract, fluoride varnish, and fluoride mouthwash, demonstrated a significant reduction in enamel surface roughness induced by bleaching. Notably, whey extract exhibited a statistically similar effect to fluoride varnish in decreasing the surface roughness of bleached enamel.

Recommendations

In-vivo studies featuring long-term follow-up are crucial to establish evidence-based guidelines for using whey extract in enamel remineralization. These studies should not only assess the effectiveness and safety of whey extract but also ensure its affordability. The goal is to develop whey extract as a cost-effective product suitable for use in clinical settings, making it an accessible and economical option for patients seeking dental treatments for enamel remineralization.

Ethics Approval and Consent to Participate

The consent to participant was not applicable. The study was conducted after approval of the research ethics committee at pharos university registration no (02-2023-8-29-3-127) on Augst 2023.

Consent for Publication

Not applicable

Availability of data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interest

The authors declare there is no conflict of interest and the research received no funding that could influence the results of this study.

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Authors' Contributions

The author's contributions are clearly stated. I.M.K was involved in the concept, design of the study, and involved in the data analysis, O.S.M, interpreted the data and performed the literature search and wrote the manuscript. Both authors reviewed and revised the manuscript critically for important intellectual content, and both authors have approved the final version of the manuscript for publication.

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