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Original Article

Ferritin Levels in Serum and Saliva of Oral Cancer and Oral Potentially Malignant Disorders

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ABSTRACT

Objective: Oral cancer remains a substantial health burden worldwide despite creditable developments in its prevention, detection, and treatment. The early detection of oral cancer offers high chances of survival and improves response to therapy making overall healthcare affordable. The aim and objective of this study were to compare and correlate serum and saliva ferritin levels in healthy subjects, oral potentially malignant disorders, and subjects with oral cancer and to assess the role of saliva as a valuable diagnostic tool.

Methods: Totally 30 participants each in 3 groups comprising healthy subjects, oral potentially malignant disorders, and oral cancer constituted the sample size. Enzyme-linked immunosorbent assay method was employed for serum and saliva ferritin levels.

Results: The respective mean serum ferritin and saliva ferritin levels were increased significantly in subjects with oral cancer (296.62 ± 82.54 ng/mL and 80.44 ± 12.94 ng/mL, respectively) and decreased significantly in oral potentially malignant disorders (69.83 ± 17.39 ng/mL and 17.49 ± 5.40 ng/mL, respectively) with a highly significant P <.001 when compared to that of healthy subjects, (116.15 ± 21.19 ng/mL and 38.47 ± 8.08 ng/mL), P <.001. All the 3 groups had a significant positive correlation between serum and saliva ferritin levels; healthy controls (r=0.622), oral potentially malignant disorders (r=0.878), and oral cancer (r=0.668).

Conclusion: The encouraging results of the present study demonstrate the potential involvement of ferritin in the pathogenesis of oral potentially malignant disorders and oral cancer. Further, the study favors saliva, as a reliable and non-invasive diagnostic tool providing a cost-effective approach for screening large populations.

Keywords: Ferritin, oral cancer, oral potentially malignant disorders, saliva, serum

INTRODUCTION

Oral cancer along with oropharyngeal cancers constitutes the 6th most common malignancy around the globe.[1] Worldwide more than 400,000 oral cancer cases are diagnosed every year, mostly in the countries such as India, Sri Lanka, Pakistan, Bangladesh, and Indonesia.[1,2] The overall 5-year survival rate of oral cancer has stayed low at 40%, but if diagnosed early the survival rates can improve up to 80%.[3] About half of all oral cancer cases are not diagnosed until in their later stages, due lack of symptoms in the early stages, and patients seeking medical help only in case of clear symptoms such as pain, growth in the mouth or surrounding areas or when lymphatic spread has taken place.[4] Oral cancer can occasionally be preceded by lesions of oral precancer which predominantly includes oral submucous fibrosis (OSMF) and leukoplakia.[5] The prevention and timely recognition of such OPMDs not only favors a decreased rate of oral cancer but also improves the chances of survival in subjects developing oral cancer[6] There is a need to develop simple, non-invasive diagnostic markers for early diagnosis, which would also aid in monitoring the progress of disease during the treatment. Recent studies have shown additional functions characterized by ferritin other than being a major iron-storage protein and these include suggestions linking ferritin to various pathways associated with cancer, such as suppressor evasion, cell proliferation, growth angiogenesis, cell death inhibition, immunomodification, immortalization, invasion, and metastasis.[7] Although several studies have assessed serum ferritin in OPMDs and oral cancer, the current study is the only till date evaluating ferritin levels of

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saliva in the aforementioned conditions. So the primary aim of this study was to assess the role of saliva as a diagnostic medium by estimating saliva ferritin levels in OPMDs and oral cancer.

METHODS

This case–control study was carried out on 90 participants reporting to the Department of Oral Medicine and Radiology of our institution, and included 3 groups (A, B, C) with 30 in each of them. Written and informed consent was obtained from all the cases before inclusion in the study. Group A comprised 30 healthy participants, group B had 30 OPMDs, and group C had 30 subjects with oral cancer. Buccal mucosa constituted the most affected anatomical site with 11 cases, followed by the tongue with 7 cases (Table 1). The OPMDs mainly consisted of 18 OSMF, 8 oral leukoplakia, and 4 cases of oral lichen planus.

Data Collection

Ethical approval was obtained from the institutional ethical committee, AB Shetty Memorial Institute of Dental Sciences (Date: October 30, 2015, Decision no: ABSM/EC 64/2015). World Medical Association (WMA)Declaration of Helsinki–Ethical Principles for Medical Research Involving Human Subjects was followed for sample collection.

Sample Collection

After obtaining institutional ethical clearance, informed consent from each subject participating in the current study was taken. The detailed case history of each subject was recorded, and the oral cavity was examined thoroughly.

Saliva Collection

Subjects were instructed not to eat, drink, or smoke for 1 hour before saliva sample collection. Each subject was seated with their head tilted forwardly. The subjects were instructed not to swallow any saliva and were abstained from speaking during sample collection time. "Spit Technique" was employed to collect unstimulated saliva from each subject participating in the study. The subjects were given a graduated container with a funnel and instructed to spit into it for 8-10 minutes. The collected sample consisted of secretions from major and minor salivary glands and gingival crevicular fluid and represented whole mouth fluid. The collected saliva sample was centrifuged (2500 rpm for 10 minutes) and the supernatant thus collected was stored at −20°C before analysis.

Main Points

- The functions of ferritin are manifold than just being an iron-storage protein.
- Although ferritin was elevated in malignancies, the level of ferritin in oral potentially malignant disorders (OPMDs) was downregulated. The more number of oral submucous fibrosis patients in OPMDs sample in this study could be the reason for this downregulation. Further large-scale studies are warranted to corroborate this finding.
- The current study verifies that saliva can be used as a safe and non-invasive diagnostic medium.

Table 1. Distribution of Oral Cancer Cases According to Their Site

Various Sites of Oral Cavity (Oral Cancer)	Number of Patients (n = 30)
Buccal mucosa	11
Tongue	7
Mandibular alveolus	3
Buccal vestibule	3
Maxillary alveolus	2
Retromolar region	1
Lip	1
Floor of the mouth	1
Buccal mucosa with skin	1

Blood Collection

The patients were seated in a comfortable position, and a syringe was used to draw 2 mL of venous blood from the antecubital vein. The blood collected was transferred into plain tubes and centrifuged for 10 minutes at 2500 rpm. Serum extracted from blood was stored in glass vials at −20°C and was later subjected to analysis.

Ferritin Enzyme-Linked Immunosorbent Assay

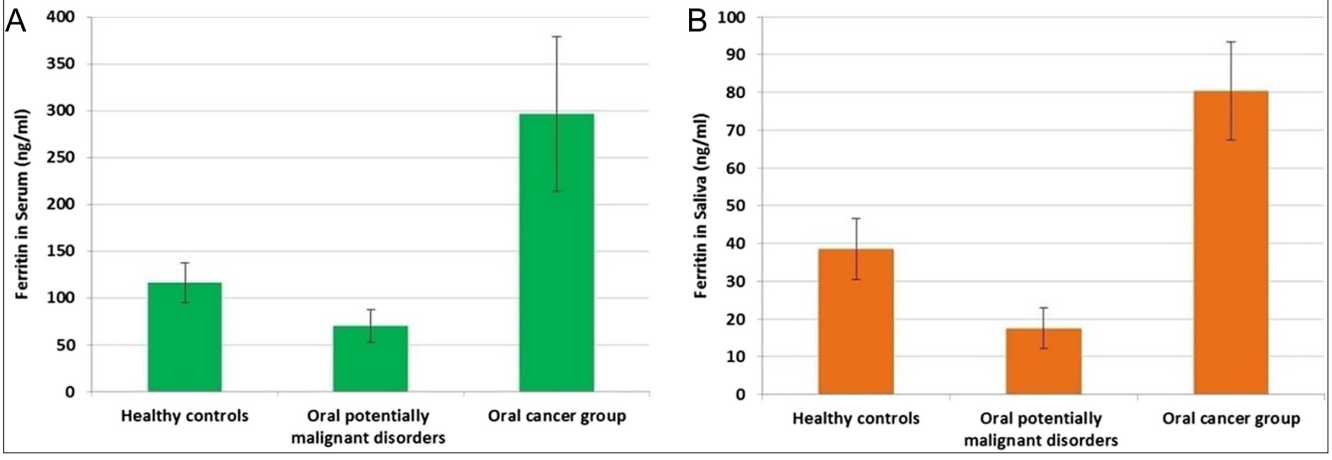
Principle of the Test

Based on a streptavidin–biotin principle, the ferritin ELISA kit involves a solid-phase sandwich assay technique. The designated wells are coated with streptavidin, and the standards, samples, and biotinylated anti-ferritin antibody reagent are added to the wells.The endogenous ferritin in the saliva and serum sample binds to the biotinylated anti-ferritin antibody at its antigenic site. The high-affinity streptavidin–biotin interaction concurrently immobilizes biotinylated antibody onto the wells. The buffer wash is used to wash off the unbound protein and excess biotin-conjugated antibody. A sandwich complex is formed, the analyte of interest lies between the 2 highly specific antibodies, and is labeled with horseradish peroxidase and biotin upon the addition of the peroxidase-conjugated anti-ferritin antibody reagent. The buffer wash is then used to wash off unbound protein and excess enzyme-conjugated antibody reagent. The intensity of the color developed upon the addition of the substrate is directly proportional to the concentration of ferritin in the samples. The color intensity relation to the concentration of ferritin is interpreted by drawing a standard curve.

Method of Analysis

Statistical Package for the Social Sciences 21.0 (IBM SPSS Corp.; Armonk, NY, USA) was used for the statistical analysis. Mean and standard deviation of the quantitative values in control and study groups were estimated. Chi-square test was used for analyzing the distribution of gender between control and study groups. One-way analysis of variance was used for the analysis of serum and salivary ferritin levels in all the groups. Tukey multiple comparison tests were used for the comparison of study groups to the control group and comparison between the study groups. Pearson correlation was used to measure the correlation between serum and saliva ferritin levels between all the 3 groups.

Figure 1. A, B. Mean serum ferritin levels in normal healthy controls, subjects with OPMDS, and subjects with oral cancer (A) and mean saliva ferritin levels in normal healthy controls, subjects with OPMDs, and subjects with oral cancer (B). OPMDS, oral potentially malignant disorders.



RESULTS

The analysis of demographic data among the 3 groups is given as follows: the age range in all the 3 groups was chosen as 20-70 years. The normal healthy controls (Group A) had mean age of 53.53 ± 10.092 years with 33.3% (10/30) females and 66.7% (20/30) males; oral potentially malignant disorders (OPMDs, group B) with a mean age of 56.87 ± 10.954 years included 46.7% (14/30) females and 53.3% (16/30) males; oral cancer (group C) had a mean age of 60.53 ± 7.300 years and comprised of 30% (9/30) of females and 70% (21/30) of males.

Mean Serum ferritin levels

Healthy controls (group A) had a mean serum ferritin level of 116.15 ± 21.19 ng/mL and group B (OPMDs) and group C (oral cancer) had mean serum ferritin levels of 69.83 ± 17.39 ng/mL and 296.62 ± 82.54 ng/mL, respectively (Figure 1A).

Mean Saliva Ferritin levels

Group A had mean saliva ferritin levels of 38.47 ± 8.08 ng/mL and group B and group C had mean saliva ferritin levels of 17.49 ± 5.40 ng/mL and 80.44 ± 12.94 ng/mL, respectively (Figure 1B).

Analysis of Statistical Significance

Serum Ferritin Levels

The mean serum ferritin levels of healthy controls (group A) was higher than group B (OPMDs) with a highly significant *P*-value of .002. Oral cancer (group C) showed higher mean serum ferritin than both group A and group B with a highly significant *P*-value of <.001 each (Table 2).

Saliva Ferritin Levels

Similarly, the mean of saliva ferritin levels was higher in group A than in group B, and the mean was higher in group C than in both group A and group B, all having a statistically significant *P*-value of <.001. (Table 3).

Pearson correlation: Significant positive correlation in ferritin levels between serum and saliva was seen in all the 3 groups;healthy controls: (r = 0.622) (Figure 2), OPMDs: (r = 0.878) (Figure 3), and oral cancer: (r = 0.668) (Figure 4).

DISCUSSION

The growth of a tumor can be monitored by evaluating tumor markers, and such evaluation can prove vital in diagnosis, staging, and future prognosis. The clinical use of a tumor marker becomes vital only when it enables its continuous measurement during a patient's clinical course after being positive for the same. The advancement or remission of malignancies can be predicted by mounting or falling values of tumor markers. The early intervention employed in high-risk OPMDs after predicting the progression of their phenotype can prevent the development of oral cancer. A considerable understanding of the various cellular processes and molecular mechanisms fundamental to

Table 2. Comparison of the Mean Difference between the Serum Ferritin of the Study Groups and the Healthy Controls, and between the Study Groups

				95% CI	
(I) Group	(J)	Mean Difference (I–J)	*P*	Lower Bound	Upper Bound
Healthy controls	OPMDs	46.32*	.002	15.40	77.23
	Oral cancer	−180.48*	<.001	−211.39	−149.56
OPMDs	Oral cancer	−226.80*	<.001	−257.71	−195.88

*The mean difference is significant at the .05 level.

Table 3. Comparison of the Mean Difference between Saliva Ferritin of the Study Groups and the Healthy Controls and between the Study Groups

(I) Group	(J)	Mean Difference (I–J)	*P*	95% CI	
				Lower Bound	Upper Bound
Healthy controls	OPMDs	20.98*	<.001	15.23	26.73
	Oral cancer	−41.97*	<.001	−47.72	−36.22
OPMDs	Oral cancer	−62.95*	<.001	−68.70	−57.20

*The mean difference is significant at the 0.05 level.

Figure 2. Correlation of serum and saliva ferritin levels in group A (healthy controls).

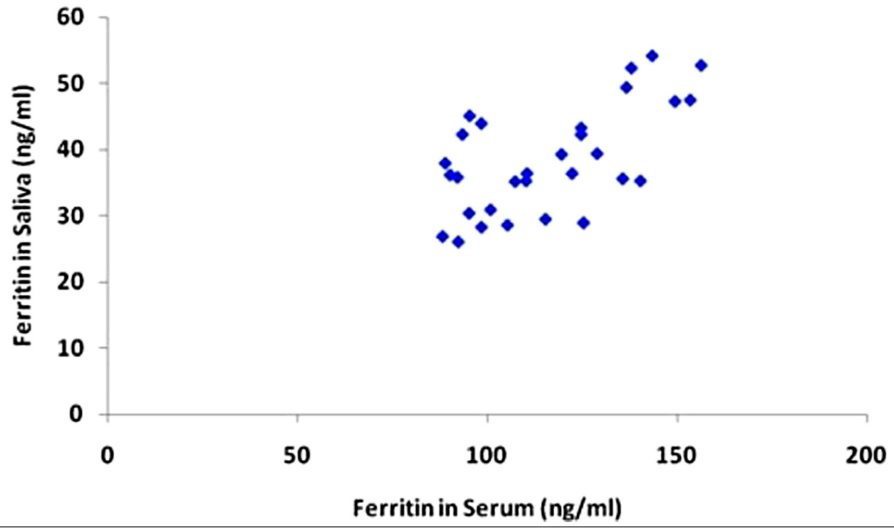
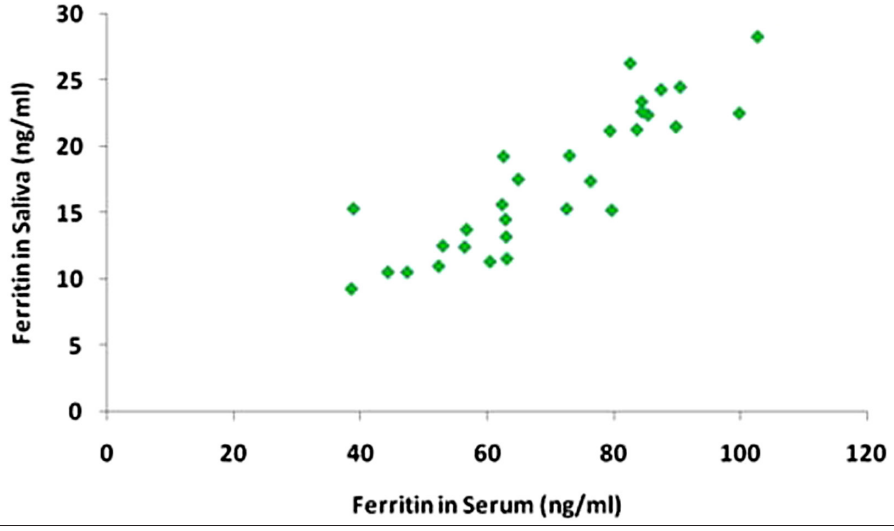
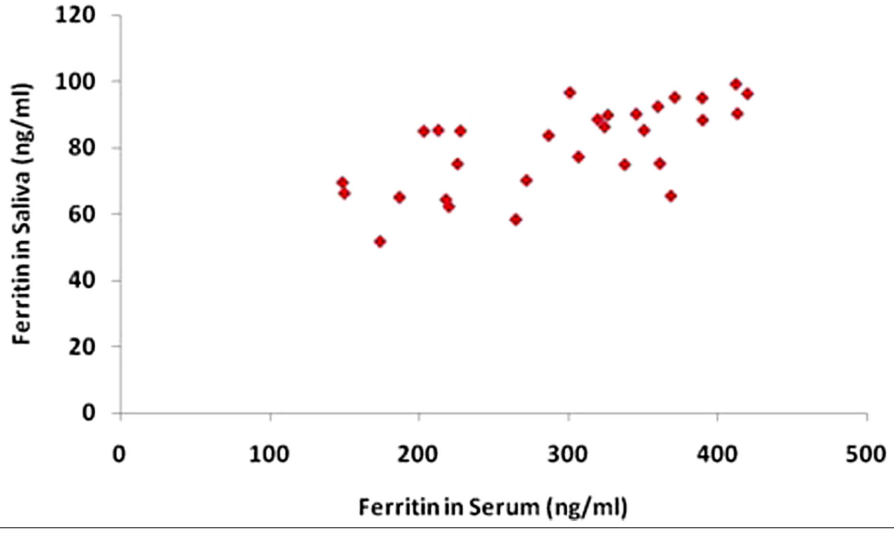


Figure 3. Correlation of serum and saliva ferritin levels in group B (oral potentially malignant disorders).



the initiation of cancer is required for the establishment of tumor markers. A special focus is given to the fact that various cellular functions can get disrupted by only a minor change in only a few regulatory proteins or genes. The tumor markers may be released as constituents in various body fluids such as serum, urine, saliva, and cerebrospinal fluid (CSF) or as substances in cells of tissues. The evaluation of tumor markers, until recently, was usually carried out in fluids other than saliva such as urine, blood, and CSF, but technological advances in the field of diagnosis have accredited saliva with certain advantages over other diagnostic media.

Figure 4. Correlation of serum and saliva ferritin levels in group C (oral cancer).



Saliva has been used for the assessment of either individual or a group of protein markers together to assist in the early recognition of oral cancer and in employing a suitable therapy. The current study was carried out with the objective of assessing the serum and saliva ferritin levels in OPMDs and subjects with oral cancer and to assess the role of saliva as a diagnostic tool.

Serum Ferritin in Oral Cancer

The statistically significant increase in serum ferritin levels in oral cancer in this study is concomitant with a cross-sectional study by Baharvand et al.[8] who evaluated serum ferritin in 60 oral cancer cases and 66 age and sex-matched controls. The elevated levels of ferritin may occur in response to chronic diseases, inflammation, and infection and may be increased despite inadequacy or deficiency of iron. Evidence gathered suggests an established role of iron in carcinogenesis.[9] Ferritin helps in maintaining the performance of important biochemical reactions and balances the process of oxidative stress.[10] Free radicals may be created as a result of increased serum ferritin, causing carcinogenesis effects. The study done by Khanna et al.[11] in conformity with our study, showed an increase in serum ferritin in oral cancer cases and confirmed much higher levels of serum ferritin in advanced stages as compared to early stages, thereby concluding that the levels of serum ferritin can be used to differentiate between early and late stages of oral squamous cell carcinoma (OSCC). Maxim et al.[12] also found increased levels of serum ferritin in head and neck cancer cases in accordance with our study and found lowered serum ferritin in cured subjects, who had no sign of clinical disease for 5 years than in those of untreated cases. Our study showed higher levels of serum ferritin in oral cancer as compared to controls, which was in accordance with studies done by Inal et al.[13] Richie et al.[14] Bhatavdekar et al.[15] Yuan et al.[16] and Vinzenz et al.[17] Elevated ferritin levels in cancer patients have been attributed to iron metabolism, hematopoiesis, and some nonspecific tissue damage. The direct secretion of ferritin from tumor cells has been postulated as the cause of the elevation of ferritin in oral cancer.

Serum Ferritin in OPMDs

Only few studies have evaluated the serum concentration of ferritin in OPMDs. Richie et al.[14] reported significantly lower serum ferritin values in oral pre-malignancies than in normal healthy subjects: the reduced ferritin level is indicative of iron deficiency, whereas raised ferritin levels can occur even in iron-deficiency states. So it can be inferred that elevated serum ferritin levels in oral cancer and low serum ferritin levels in oral precancerous lesions of oral cavity have different underlying causes. Thakur et al.[18] found a significant decline of serum ferritin in OSMF patients as compared to normal healthy controls and further showed a progressive decrease in serum ferritin levels as the histopathological grade increased. The reduced serum ferritin in OSMF can be related to the corollary of increased utilization of iron in OSMF for collagen synthesis. Thus, as the iron stores get depleted, serum ferritin level decreases and is downregulated. In the current study, serum ferritin levels in OPMDs were significantly reduced in OPMDs, and as the subjects were from a similar socioeconomic background, the decreased levels of serum ferritin could be because of the disease process itself rather than being a cause. Thus, it can be stated that the lack of proper intake of micronutrients in the diet leads to anemia initially, which worsens later due to the progression of the OPMDs. As low ferritin is one of the diagnostic criteria for iron deficiency, the increased iron utilization in case of OPMDs (as in OSMF) could be the reason for decreased levels of ferritin.

Saliva Ferritin Levels in Oral Cancer and OPMDs

The existing literature showed no earlier study employing saliva ferritin levels in oral cancer and OPMDs. In the present study, the mean saliva ferritin level in subjects with oral cancer showed a significant elevation (similar to that of serum) when compared with the mean saliva ferritin levels of normal healthy subjects. Although it is not precisely known how tumor markers manifest in saliva, they may be either derived from serum or can be produced locally. When derived from serum, ferritin in saliva can

appear as a constituent of normal saliva composition, active transport, passive diffusion, an outflow of crevicular fluid, and ultrafiltration through tight junctions. When produced locally, it can be a result of cell necrosis, apoptosis, active release, or trauma. Thus, the increase in saliva ferritin levels in oral cancer subjects could be due to direct leakage from the malignant tumors in addition to its derivation from serum, as ferritin has been considered to be a product of damaged cells.

The saliva ferritin levels in OPMDs were significantly lower than saliva ferritin levels of healthy controls. The decreased saliva ferritin levels in OPMDs could be due to increased utilization of iron, as comparable results were seen in serum ferritin levels of OPMDs. The altered epithelial turnover rate, decreased intake of micronutrients owing to the difficulty in mastication, and increased utilization of iron as seen in subjects with OSMF to produce collagen all lead to iron depletion in OPMDs. These factors, in turn, could lead to the reduction of saliva ferritin levels in OPMDs.

Limitations of the Study

The analysis of ferritin was not interpreted according to the stages in the oral cancer study group. The sample size in each group was fairly small.

CONCLUSION

The current study showed a significant increase in serum and saliva ferritin levels in oral cancer and a significant decrease in serum and saliva ferritin levels in subjects with OPMDs thereby indicating that ferritin can be used as an adjunctive diagnostic biomarker in both oral cancer and OPMDs. Furthermore, the positive significant correlation between serum and saliva ferritin levels signifies that saliva can be utilized as a reliable, non-invasive tool for diagnosing and monitoring of OPMDs and oral cancer. Saliva, which is a readily available sample containing a substantial number of proteins and peptides, is used as a biomarker for diagnosing various oral and systemic diseases. It is one of the most reliable tools for diagnosing oral squamous cell carcinomas because of its direct contact with oral cancer and OPMDs. Thus, saliva can be used in a non-invasive fashion for the diagnosis of OPMDs and oral cancer subjects, which has the potential to dramatically reduce anxiety and discomfort associated with blood sampling procedures and increases the willingness of patients to undergo frequent health inspections.

Ethics Committee Approval: This study was conducted at NITTE (Deemed to be University), AB Shetty Memorial Institute of Dental Sciences, Mangalore- Karna taka- India from January 2016 to December 2017 and was approved by the institutional ethics committee (Date: June 30, 2017, Decision no: ABSM/EC64/2015).

Informed Consent: Informed consent was taken from all the volunteers, after having been informed of the study details and provided with clarifications.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – S.A.B., S.G.B.; Design – S.A.B., S.G.B., R.L.C.; Supervision – S.G.B., R.L.C.; Materials – S.A.B., D.S.P., S.B., U.L.D.; Data Collection and/or Processing – S.A.B., U.L.D.; Analysis and/or Interpretation – S.A.B., R.L.C., S.B., U.L.D.; Literature Review – S.A.B., D.S.P., S.B.; Writing – S.A.B., D.S.P.; Critical Review – S.G.B., R.L.C.

Declaration of Interests: There are no conflicts of interest.

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