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Lipid Analysis For Atherogenic Indices Assessment In Periodontal Disease: Using A Point Of Care Testing (POCT) Device – An Epidemiological Study.

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Abstract

Background: Cardiovascular diseases (CVDs) are the leading cause of death globally. Screening the population for risk factors for CVD is one mechanism used by various countries in the Region to identify and address the CVD burden. However, population-level screening for CVD risk factors has no effect on lowering CVD morbidity and mortality . POCT devices offer a potential alternative to traditional venous blood collection, as novel care pathways for early detection of CVDs. They are in concordance with laboratory testing the potential use of these devices in dental setting provides an opportunity for screening the individuals "at CVD risk". Stronger evidences suggesting emerged risk of CVDs in periodontal patients, but it was still insufficient for periodontitis to be classified as a causal risk factor. The present study objectives are to establish evidence about the benefits of POCT devices in dental setting and to explore the lipid levels and atherogenic index of periodontal disease patients visiting our institution.

Materials and Methods: This is a single-center, descriptive cross sectional, collaborative study of Biochemistry and Periodontology in a dental hospital. We have included the age ≥18 - 50 years we excluded smokers, alcoholics, patients with known history of hypertension, diabetes, CVD and thyroid disorders and patients on medication like antihypertensive or lipid lowering drugs. Non- probabilistic convenience sampling will be applied to enroll participants, consecutively according to their convenience. The sampling process will be continued for two months from the date of commence.

Results: The association of atherogenic indices with different age groups was evaluated using chi square test. No significant association of AIP, CRI II and AC with age was found. While CRI I and Non HDL-c were association with age.

Conclusions: Castelli's index I and II are very useful for the identification of cardiovascular risk. Individuals suffering with Periodontitis, POCT devices are very helpful and feasible technique to assess the lipid parameters and Atherogenic indices.

Keywords: HDL, LDL, CVD, CRI I, CRI II, PCOT.

Introduction

Cardiovascular diseases (CVDs) are the leading cause of death globally. An estimated 17.9 million people died from CVDs in 2019, representing 32% of all global deaths. Of these deaths, 85% were due to heart attack and stroke. Over three quarters of CVD deaths take place in low- and middle-income countries. Out of the 17 million premature deaths (under the age of 70) due to non-communicable diseases in 2019, 38% were caused by CVDs. Most cardiovascular diseases can be prevented by addressing behavioral risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity and harmful use of alcohol. It is important to detect cardiovascular disease as early as possible so that management with counselling and medicines can begin. Screening the population for risk factors for CVD is one mechanism used by various countries in the Region to

identify and address the CVD burden. However, population-level screening for CVD risk factors has no effect on lowering CVD morbidity and mortality [1, 2]

Rationale of screening and screening strategies: Many patients do not have any symptoms of CVD before a serious first event, such as MI or stroke. Early detection of people at increased risk for CVD is an important part in prevention and health promotion. The conventional risk factors such as hypertension, diabetes mellitus dyslipidemia, smoking, obesity are believed to be associated with increased prevalence of CAD in Indians [3]. The screening strategies still fall far short of the established coverage thresholds in both rural and urban settings of India [4].In India, the growing incidence of CVDs is not yet seen as a public health challenge which has resulted in the absence of a strong policy targeting them. Though the Government has recently started taking initiatives by introducing programmers' focusing on screening and detection of the rural population for symptoms of CVDs, these initiatives need to be scaled up to reach out to the population all over the country. A focused policy targeting CVDs will lead to concentrated efforts to reduce the mortality and morbidity arising due to them [5]. Advanced medical technology has raised the cost of CVD screening at primary level. The issue of affordability is further magnified by the low penetration of health care screening facilities in India [5].

Rationale of the study: Suboptimal level health care facilities, trained personnel and standard laboratories for the early screening of CVDs [6] are the critical gap in the health facilities in India. These gaps highlight need for strengthening of primary health care systems for scaling up of interventions for screening and management of CVDs so as to achieve goal of reduce the burden of related mortality and morbidity. Expanding CVDs screening to dental offices provides a new screening strategy to fill part of the gap in primary care services. Previous studies have reported dentists' willingness to provide cardiovascular disease screening and the feasibility of providing cardiovascular disease screening in the dental care setting [7]. POCT devices offer a potential alternative to traditional venous blood collection, as novel care pathways for early detection of CVDs. They are in concordance with laboratory testing [8] the potential use of these devices in dental setting provides an opportunity for screening the individuals "at CVD risk". Stronger evidences suggesting emerged risk of CVDs in periodontal patients, but it was still insufficient for periodontitis to be classified as a causal risk factor. Most of the studies assessed 10 years risk prediction chart for screening CVD risk in periodontal patients using standard biochemical methods [9, 10]. But, studies were not focused on assessment atherogenic indices in periodontal patients. Therefore, we designed a study to assess the atherogenic indices of apparently healthy periodontal patients using POCT devices. This study will provide documented evidence about the use of POCT devices and atherogenic indices as feasible screening tools in a dental setting to overcome the suboptimal health care CVD screening facilities in India. The objectives of present study were to estimate the atherogenic indices in periodontal patients using a point of care testing (POCT) device, to establish evidence about the benefits of POCT devices in dental setting and to explore the lipid levels and atherogenic index of periodontal disease patients visiting our institution.

Materials and Methods

This is a single-center, descriptive cross sectional, collaborative study of Biochemistry and Periodontology in a dental hospital. Inclusion criteria were 1) Age ≥18 - 50 years 2) accepted to give written informed consent. Exclusion criteria were; 1) Smokers: defined as a person who smoked more than one cigarette on more than 1 day for the past 30 days 2) Alcoholics: defined as a person who consumed more than one drink containing alcohol in the past 30 days. [8] 3).Patients with known history of hypertension, diabetes, CVD and thyroid disorders 4) Patients on medication like antihypertensive or lipid lowering drugs. Non- probabilistic convenience sampling will be applied to enroll participants, consecutively according to their convenience. The sampling process will be continued for two months from the date of commence. Baseline characteristics of participants like age, gender and history related their health was collected through a questionnaire. After applying selection criteria eligible candidates were selected. Study protocol and its benefits were explained to the selected; and informed consent was collected. Periodontitis was examined the patient and categorize based on their periodontal disease stage. Then participants were re-explained about the study procedure and asked to revisit the Periodontology department on next day morning in fasting state. However, PD treatment will be continued.

Periodontal examination: After obtaining consent of the participant periodontal assessment was done using the following parameters and score was given: 0 for normal gingival, 1 for Mild inflammation slight change in color slight edema. No bleeding on probing, 2 for Moderate inflammation – redness, edema and glazing. Bleeding on probing present, 3 for Severe inflammation – marked redness, edema and ulceration. Tendency to spontaneous bleeding. All these assessments were carried out with the UNC-15 periodontal probe. Individuals with early stage of gingivitis were categorized into stage 1 periodontitis.

Periodontal examination: After obtaining consent of the participant periodontal assessment was done using the following parameters and score was given: 0 for No plaque in the periodontal area, 1 for A film of plaque adhering to the free periodontal margin and adjacent area of the tooth, 2 for moderate accumulation of soft deposits within the periodontal pocket, 3 for Abundance of soft tissue matter within the periodontal pocket. All these assessments were carried out with the UNC-15 periodontal probe. Individuals with early stage of gingivitis were categorized into stage 1 periodontitis.
PD measurements were obtained at six sites (mesio buccal, mild buccal, disto buccal, medio lingual, mid lingual, disto lingual) by using UNC-15 periodontal probe. The PD was measured from the free gingival margin (GM) to the base of the pocket. The probe was maintained parallel to the long axis of the tooth at eh mid buccal and mid lingual sites, but at the proximal sites, the probe was placed as close to the contact point as possible and slightly angled to determine the apical most extent of the pocket.
CAL was determined by measuring the distance from the cement-enamel junction (CEJ) to the base of the gingival sulcus with a UNC-15 periodontal probe. This can be summarized as: when the gingival margin (GM) coincides with the CEJ, the loss of attachment equals the pocket depth (PD). When the gingival margin is located on the anatomical crown, CAL=PD-CEJ to GM. When the gingival margin is located apical to the CEJ, CAL= PD+CEJ to GM (all measurement s are in millimeters). CAL was measured at 6 sites on each tooth: mesio buccal, mild buccal, disto buccal, medio lingual, mid lingual, disto lingual.
G-Care Lipid Analyzer: GCare Lipid Analyzer is a compact POC device to measure lipids in capillary and venous blood. The GCare Lipid Profile Test Strip will be inserted into the inlet at the bottom of the device. From the participants who revisited the department, approximately 40µl of capillary blood will be drawn by fingerstick sampling and collected into a capillary tube without air bubbles and dropped onto lipid test strip. After 180 seconds, total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) will be noted by clicking the arrow button on the device serially. The LDL-C value will be calculated according to the Fried Ewald formula (TC - HDL-C TG/5) when the TG value is <350 mg/dl.

Atherogenic index was calculated using following formulae:
1. Atherogenic index of plasma (AIP) = log triglyceride/high-density lipoprotein cholesterol (HDLc)
2. Castelli's Risk Index (CRI-I) = Total cholesterol/HDL-C
3. CRI-II = Low density lipoprotein cholesterol/HDL-C
4. Atherogenic coefficient (AC) = (Total cholesterol−HDL-C)/HDL-C
5. Non-HDL-C (NHC) = Total cholesterol−HDL-C.

Comparisons of data was done among age groups [Group-1: 18 -35 years; Group-2: 36-50 years], PD stage [stage-1 initial; stage-2 moderate; stage-3 severe; stage-4 severe with loss of teeth] and between genders [male and female].
Statistical analysis: Data recording and processing was performed using Microsoft Excel. The data analyzed statistically using Mean, standard deviation, student's t-test and percentages. Pearson correlation coefficient was applied to assess association between the variables.

Results
The weighted characteristics of the analyzed samples are shown in Table 1. The final sample included 105 participants, 56 (52.1%) men, and 49 (47.7%) women, aged 18– 50 years, and may representing adults who were visiting our dental hospital during the period from March 2023 to November 2023. Mean age of the participant is 31.10±4.96. A higher percentage of participants (86.2%) were educated beyond secondary grade level. Total 78.2% participants were from rural background. Mean BMI of the participants was 23.22±3.26; total 29 men and 32 women were with BMI >25 i.e., obesity. A higher frequency of participants with periodontitis had decreased HDL-c levels followed by increased Triglycerides, LDL-c and total cholesterol. These lipid alterations were high in males compared to females. Among the individual lipid parameters only triglycerides (OR=3.355) were found to be independently associated with periodontitis. Other lipids CHO (OR=1.3889) HDL-c (OR=2.029) LDL-c (OR=1.35) and an anthropometric parameter increased BMI (OR=0.5706) were not associated with periodontitis independently in multinomial logistic regression analysis.

Results indicated that mean value of cholesterol 174.39±28.90; triglycerides 127.07±52.59 and non HDL-c 118.62±26.12 were in normal allowable limits. While HDL-cholesterol 46.03±8.99, and LDL-cholesterol 105.41±26.51 were below and above the allowable limits respectively. Atherogenic index

of plasma AIP>0.24, Catelli index-I >5, Catelli index-II >3 Atherogenic coefficient >0.11 and Non HDL-c >120mgl/dl were considered a cardiovascular risk factor. The significance of atherogenic indices to identity CVD risk in different stages of periodontitis was evaluated with chi-square test. (Table 1)

Table 1: Association of Atherogenic indices with CVD risk in periodontitis

Atherogenic index	**Severity of periodontitis**	**No. of participants with CVD risk**	**No. of subjects with no CVD risk**	**Significance test**
Atherogenic index of plasma CVD risk ≥0.24 vs no CVD risk <0.24	Initial	6	46	χ2= 7.5079 n= 105 p=0.023425
	Moderate	12	23	
	Severe	7	11	
Castelli's Risk Index-I CVD risk ≥5.0 vs no CVD risk <5.0	Initial	4	48	χ2= 18.976 n= 105 p=000076
	Moderate	16	19	
	Severe	8	10	
Castelli's Risk-II CVD risk (≥3.0) vs no CVD risk (<3.0)	Initial	17	35	χ2= 15.3036 n= 105 p=0.05
	Moderate	26	9	
	severe	11	7	
Atherogenic coefficient CVD risk ≥0.11 vs no CVD risk <0.11	Initial	18	34	χ2= 0.4889 n= 105 p=0.783118
	Moderate	10	25	
	Severe	5	13	
Non HDL-c >120 mg/dl Vs <120mg/dl	Initial	24	28	χ2= 2.5555 n= 105 p=0.278658
	Moderate	16	19	
	Severe	12	6	

The association of atherogenic indices with different age groups was evaluated using chi square test. No significant association of AIP, CRI II and AC with age was found. While CRI I and Non HDL-c were association with age.

Association of age with different atherogenic indices in periodontitis with CVD risk

Parameter	**Age group**	**Low – high risk CVD risk**	**No CVD risk**	**χ2-value**	**p-value**
AIP	18-35	8	39	2.3997	0.121361
	36-50	17	41		
CRI- I	18-35	11	36	3.5951	0.057949*
	36-50	17	41		
CRI- II	18-35	29	18	2.3929	0.12189
	36-50	25	33		
Atherogenic coefficient	18-35	12	35	1.3728	0.241334
	36-50	21	37		
Non HDL-c	18-35	31	16	9.1923	0.00243*
	36-50	21	37		

*significant

Most of the participants were with increased CRI- II followed by non HDL-c, increased AC, increased CR-I and AIP.

Discussion

In the current study, we evaluated different atherogenic indices in apparently metabolically healthy periodontitis patients. Castelli's index I & II and Atherogenic index of plasma were found to be significant, convenient, independent, and better predictor for CVD risk in periodontitis patients. While, it has been observed that atherogenic coefficient and non-HDL cholesterol were not in association with CVD risk in periodontitis. Age and gender were not the significant factors in this elevation of atherogenic indices.

In the present study overall 80% of participants were with decreased HDL cholesterol level followed by increased TGL and LDL cholesterol. As there is no significant differences in the lipid levels by gender this elevated lipid traid is the major risk factor for the development of CVD in periodontitis patients. Studies have shown that patients with periodontitis have significantly lower HDL-C.[11,12] A meta-analyses also showed that participants with periodontitis had lower HDL-C levels than those without the condition. Other studies have, however, found no significant correlation between periodontitis and HDL-C. The heterogeneity of these results could be attributed to differences in sample size and participant characteristics.[13]. Comprehensive lipid levels have been measured using AIP, which is a valid biomarker of dyslipidemia [14]. Numerous biological occurrences, such as obstructive sleep apnea, menopausal women's bone trabecular scores, and even death in patients with newly installed coronary arteries, have been linked to AIP, according to studies [15,16,17]. It is favorably correlated with the risk of cardiovascular illnesses and is also thought to be a biomarker for metabolic syndrome and coronary syndrome [18]. We deduced that periodontitis and AIP could be related based on the facts provided above. The conventional small-sample, single-center population method, which has limited predictive value, is still debatable when assessing the connection between lipid levels and periodontitis. Our results shown AIP was not correlated with CVD outcome in periodontitis.

Total cholesterol/HDL ratio, sometimes referred to as the Castelli index or atherogenic index, was thought to be a significant factor and an indication of vascular risk [19], with a predictive value higher than that of the individual measures. While lower HDL cholesterol concentration was linked to many risk factors, including elements of the metabolic syndrome, and likely includes independent risk, higher total cholesterol concentration was demonstrated to be an atherogenic lipid marker [20]. No study focused on Castelli index as a risk factor for CVD risk in periodontitis. A lower HDL-C concentration ($P = 0.048$) and a more unfavorable Castelli's risk score were observed to be associated with increased CAL > 5 mm as opposed to CAL ≤ 5 mm. When assessing dyslipidemia, it's interesting to note that compared to RA patients in remission (18.2%, $P = 0.025$), more than twice as many active RA patients (42.2%) had a TC concentration > 6 mmol/L. Nevertheless, only 19.2% and 6.4% of the RA patients had high LDL-C and lowered HDL-C values, respectively, whereas over one-third of the patients had elevated TC concentrations. Our research revealed that individuals with periodontitis had a higher correlation between their risk of CVD and the Castelli index I and II, regardless of the specific lipid parameters[21].

This study is the first attempt to use several atherogenic indices to screen for CVD risk in periodontitis. When it comes to allocating resources, designing health interventions, and gauging the success of such programs in low- and middle-income nations like India, the assessment of a population's CVD risk is preferable to the evaluation of individual risk factors. Assessing the risk of CVD does not benefit from the estimate of specific lipid parameters. When periodontitis sufferers use the Castelli index I and II, it can provide a reliable indicator of their risk for CVD. Those with low and high risk of developing a heart attack were studied to examine the individual and adjusted impacts of the risk variables (physical activity, alcohol, family history, BMI, and poor diet) that are not included in the WHO risk algorithm . There were certain restrictions on this study. It is possible that the risk factor evaluation in a screened sample may not accurately represent the real prevalence in the community, which is best addressed by random sampling. As a result, the prevalence of risk factors might be exaggeratedly high. Moreover, the population examined comprised just 5% of the eligible age group that visited our facility. It is also recognized that under some circumstances, the atherogenic indices may understate the true risk of CVD.

Conclusion: Castelli's index I and II are very useful for the identification of cardiovascular risk. Individuals suffering with periodontitis, POCT devices are very helpful and feasible technique to assess the lipid parameters and Atherogenic indices.

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