

# Monocyte-to-High-density Lipoprotein Cholesterol Ratio as a Novel Inflammatory Marker in Periodontal Disease: A Pilot Study

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## ABSTRACT

**Aim:** The monocyte-to-high-density lipoprotein cholesterol ratio (MHR) has currently been proposed as an indicator of inflammation. The aim of the present study was to assess the relationship between the monocyte-to-high-density lipoprotein cholesterol ratio and periodontal health and disease.

**Materials and methods:** A total of 90 patients were selected for the study – 30 healthy patients (group I) and 60 periodontitis patients (groups II and III). All the patients were subjected to blood sampling and serum malondialdehyde (MDA), high-density lipoprotein cholesterol (HDL) levels and monocyte counts were estimated.

**Results:** Monocyte-to-high-density lipoprotein cholesterol ratio was  $80.64 \pm 28.71$  for patients with moderate periodontitis (group II),  $95.14 \pm 53.21$  in severe periodontitis (group III), and  $14.28 \pm 16.05$  for the healthy patients. Monocyte-to-high-density lipoprotein cholesterol ratio values were found to be statistically significantly higher than the control group ( $p < 0.001$ ). Monocyte-to-high-density lipoprotein cholesterol ratio also showed significantly positive correlation with the severity of periodontitis.

**Conclusion:** Malondialdehyde and MHR are increased in periodontal disease and correlate with severity of the periodontal disease.

**Clinical significance:** Monocyte-to-high-density lipoprotein cholesterol ratio is a novel, readily available inflammatory and oxidative stress marker in patients with periodontitis and can be useful to evaluate periodontitis and disease severity.

**Keywords:** Biomarker, High-density lipoprotein cholesterol, Inflammation, Malondialdehyde, Monocytes, Periodontitis.

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## INTRODUCTION

Periodontal disease is initiated by periodontopathic bacteria but perpetuated by inflammation, leading to bone loss and eventually tooth loss. Inflammation seems to be the cardinal and common phenomenon in the pathogenesis of periodontal disease and it also links various systemic diseases to periodontal disease. This inflammation is kick-started by components of biofilm bacteria such as lipopolysaccharide, which triggers a series of immune and inflammatory mechanisms. However, in a healthy individual, resolution ensues and the offending organisms are cordoned off. However, if the offending attack is overwhelming or the host immune system is compromised, the array of pro-inflammatory cytokines from cells like neutrophils and macrophages along with the matrix metalloproteinases promotes the bystander tissue damage that finally leads to bone and tooth loss.<sup>1</sup> Moreover, the milieu in periodontal disease tips toward oxidative distress rather than oxidative stress. Reactive oxygen species (ROS) are generated in multiple compartments within the cell. Oxidative stress due to excessive production or due to defective neutralization leads to ROS-mediated damage through oxidation of cellular constituents, including nucleic acids, lipids, and proteins. Reactive oxygen species-mediated lipid peroxidation processes cause break in the cell membrane lipid bilayer, leading to inactivation of membrane-bound receptors and enzymes, and increase in membrane permeability. Malondialdehyde is one of the end products of lipid peroxidation and its levels are increased in patients with periodontitis.<sup>2</sup> Monocytes are the largest among the leukocytes, make up 3–7% of the total cells,

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and are generally the second cell type to move to the site of inflammation. Monocytes differentiate into macrophages in tissues and like neutrophils, they can eradicate the offending pathogens and clear the debris by phagocytosis. However, the accumulation of monocytes exacerbates oxidative stress and inflammation. Moreover, activated monocytes, macrophages, and fibroblasts produce cytokines within periodontal lesions, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ),

and interleukin-6 (IL-6).<sup>3</sup> In contrast, HDL, a complex molecule, has potent anti-inflammatory effects – decreasing activation of monocytes and neutrophils, inhibiting leukocyte adhesion, and also has antithrombotic effects,<sup>4</sup> thus protecting against cardiovascular disease. A number of environmental factors can modify HDL resulting in a defective or impaired molecule, altering its beneficial effects. Such a scenario exists in many chronic inflammatory conditions/diseases like cardiovascular disease, hypercholesterolemia, and diabetes mellitus. An array of studies has studied the relationship between dyslipidemia and periodontal disease and a subset of studies have reported reduced levels of HDL in subjects with periodontitis.<sup>5–7</sup> Recently, a relation between a low HDL level and a high monocyte count has been described. Monocyte-to-HDL ratio is now considered as an early marker/predictor in many chronic inflammatory diseases like cardiovascular disease, polycystic ovarian disease, chronic kidney disease, metabolic syndrome, etc. To date, no study has explored the association between periodontitis and the MHR. Therefore, the aim of this pilot study was to estimate the levels of MDA and MHR in health and periodontal disease (moderate and severe), and also to correlate their levels with severity of periodontal disease.

## MATERIALS AND METHODS

This was a single-centered study, and the patients were recruited from the OP Department of Periodontics, Thai Moogambigai Dental College and Hospital, Chennai from January 2019 to March 2019. Ethical clearance was procured from the Institutional Review Board, the study purpose as well as the details were explained, and informed consent was obtained from patients undergoing this study. A total of 90 patients were selected for the study. All participants were between 25 and 58 years old – 30 healthy patients (group I) and 60 periodontitis patients (groups II and III). Periodontal health was diagnosed based on the absence of bleeding on probing and absence of pockets. Periodontitis was diagnosed as per the 2017 AAP Classification of Periodontal Disease.<sup>8</sup> Group II consisted of 30 patients, with stage-II grade-B periodontal disease (moderate periodontitis), and group III included 30 patients with stage III grade B periodontal disease (severe periodontitis with potential for additional tooth loss). A single examiner with calibrated Williams periodontal probe carried out the examination. The presence of interdental clinical attachment loss at  $\geq 2$  nonadjacent teeth, or buccal/oral clinical attachment loss  $\geq 3$  mm with pocketing  $\geq 3$  mm at  $\geq 2$  teeth, was taken into consideration for identification of periodontitis. The presence of cardiovascular disease, diabetes, chronic lung disease, liver diseases, malignancies, other endocrine disorders, renal dysfunction, alcoholism, and subjects on lipid-lowering drugs were considered as exclusion criteria for participation in this study.

### Blood Sampling

Following diagnosis, patients were subjected to blood sampling. A nonfasting blood sample of 10 mL was collected from all the patients via venipuncture of the right/left arm from the antecubital vein using a 21-gauge sterile syringe and processed for monocyte counts and HDL levels following the standard pathology protocol. Serum MDA levels were estimated by the method previously described by Yagi<sup>9</sup> and expressed as  $\mu\text{M/L}$ . To obtain the reaction product of malonaldehyde with TBA, 1,1,3,3-tetraethoxypropane

was used.<sup>4</sup> One nanomole of tetraethoxypropane dissolved in 4.0 mL of distilled water was mixed with 1.0 mL of TBA reagent (0.67% TBA aqueous solution + glacial acetic acid, 1: 1, v/v), heated at 95°C for 60 minutes. The reaction product had an absorption peak at 532 nm and fluorescence green. The excitation and emission spectra were measured using a spectrofluorophotometer.

The HDL levels were estimated using HDL Direct from Diatek (Diatek Healthcare Pvt. Ltd., Kolkata) and were analyzed using a sophisticated semi-auto clinical chemistry analyzer (Erba Manheim Chem 5 Plus v2, Erba Diagnostics Mannheim GmbH, Germany). Monocyte count was calculated by multiplying white blood cell percentage by total white blood cell count. Monocyte-to-high-density lipoprotein cholesterol ratio was calculated by dividing monocyte count into HDL.

### Statistical Analysis

Statistical analyses were performed using SPSS software, version 21 (IBM Corp.) for Windows. Initially, normality was assessed for the collected data. It was found to be normally distributed, and hence parametric tests were followed for the study. Mean and standard deviations were used to express continuous variables, and categorical variables were expressed as counts and percentages. Significance level was fixed at 0.05. The intergroup comparison between the healthy, moderate periodontitis group, and severe periodontitis group was calculated for HDL, MHR, MDA, and monocyte using analysis of variance (ANOVA). The power of the study was estimated to be 95% using G power 3.1.9.2 statistical tool.

## RESULTS

The demographic characteristics and the baseline values are given in Table 1. The total number of subjects was 90, of which 30 were healthy subjects. In the healthy group (group I), the mean age was  $36 \pm 10.9$ , of which 73.3% were females and 26.7% were males. The periodontitis group had 60 subjects, among which  $44.8 \pm 8.5$  belonged to moderate periodontitis (group II), and  $43.3 \pm 11.2$  in the severe periodontitis category (group III). The moderate periodontitis (group II) had 53.3% females and 46.7% males. Among the severe periodontitis (group III), 66.7% were females and 33.3% were males. Table 2 depicts the comparison of the serum MDA, monocytes, HDL levels, and MHR ratio among the periodontitis group (groups II and III) and the healthy control group (group I). The observed values for the MDA levels in the healthy subjects and the two groups of periodontitis patients were  $0.08 \pm 0.01$ ,  $1.06 \pm 0.27$ , and  $1.37 \pm 0.52 \mu\text{M/L}$ , respectively. Differences in the MDA levels of patients with moderate and severe periodontitis in comparison with those

**Table 1:** Demographic table

Variables	Healthy	Moderate periodontitis	Severe periodontitis
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Age	$36 \pm 10.9$	$44.8 \pm 8.5$	$43.3 \pm 11.2$
	(%)	(%)	(%)
Female	73.3%	53.3%	33.3%
Male	26.7%	46.7%	66.7%

Values are presented as mean  $\pm$  standard deviation. SD, standard deviation; (%), percentage

**Table 2:** Comparison of the serum MDA, monocytes, HDL levels, and MHR ratio among healthy group (group I) and periodontitis group (groups II and III)

Variables	Healthy (Group I)	Moderate periodontitis (Group II)	Severe periodontitis (Group III)	LOS*
	Mean ± SD	Mean ± SD	Mean ± SD	
Monocyte	0.80 ± 0.93	2.71 ± 0.85	3.1 ± 1.04	<0.001 <sup>a</sup>
HDL	54.1 ± 14.89	36.26 ± 11.15	36.46 ± 20.2	0.004 <sup>b</sup>
MHR	14.28 ± 16.05	80.64 ± 28.71	95.14 ± 53.21	<0.001 <sup>a</sup>
MDA	0.08 ± 0.01	1.06 ± 0.27	1.37 ± 0.52	<0.001 <sup>a</sup>

\*Significant ( $p \leq 0.05$ ). HDL, high-density lipoprotein cholesterol; LOS, level of significance; MHR, monocyte-to-HDL ratio; MDA, malondialdehyde.

<sup>a</sup>Statistically highly significant. <sup>b</sup>Statistically significant

of healthy subjects were also noted, which was highly statistically significant ( $p \leq 0.0001$ ). The monocyte levels also increased from health to periodontitis,  $0.80 \pm 0.93$  (group I),  $2.71 \pm 0.85$  (group II), and  $3.1 \pm 1.04$  (group III), and statistical significance was noted. On analyzing the HDL values among the groups, it was also statistically significant ( $p \leq 0.05$ ) as elevated values were observed in the periodontitis group i.e.,  $36.26 \pm 11.15$  (group I),  $36.46 \pm 20.2$  (group II) compared with group I ( $54.1 \pm 14.89$ ). However, significance was not noted between moderate and severe periodontitis. The MHR in healthy group (group I) was  $14.28 \pm 16.05$ ,  $80.64 \pm 28.71$  in group II, and  $95.14 \pm 53.21$  in group III. Highly significant ( $p \leq 0.001$ ) difference between the health and periodontitis variables was perceived for monocytes and MHR. From the results, it was inferred that MDA and MHR are increased in periodontal disease and correlated with the severity of the periodontal disease.

## DISCUSSION

Periodontal disease, a common inflammatory disease, initiated by a dysbiotic biofilm and subsequently propagated by the host immune system, results in destruction of investing structures of the teeth and also has a potential impact on systemic well-being. In conjunction with the bacterial challenge, the host's immune response plays an important role in the onset and progression of periodontitis. Activated monocytes, macrophages, and fibroblasts produce cytokines within periodontal lesions, including TNF- $\alpha$ , IL-1 $\beta$ , and IL-6.<sup>10</sup> Oxidative stress is integral in periodontal disease, and one of the key features in development and progression of periodontal disease. During inflammation, ROS production is dramatically increased, predominantly by polymorphonuclear lymphocytes, monocytes, and macrophages during respiratory burst phenomenon,<sup>11</sup> which overwhelms the antioxidant system, leading to tissue damage. In addition to the indirect damage caused by ROS, oxidative stress can cause disruptions in normal mechanisms of cellular signaling. Several biomarkers can be assessed to reflect the oxidative stress state of the tissue. However, due to shorter dwindling lifespan of ROS, it is a formidable challenge. Of the widely investigated ROS markers in periodontitis, lipid peroxidation products produced *in vivo*, such as MDA, 4-hydroxy-nonenal (HNE), and isoprostane, have been considered for both local and systemic burden associated with periodontitis. Malondialdehyde is technically easier to quantify and has proven to be a valid clinical marker, though it does not have any functional impact on the pathogenesis of periodontal disease. Wei et al.<sup>2</sup> found higher levels of MDA in gingival crevicular fluid,

saliva, and serum of patients with chronic periodontitis compared with controls. Also, Ghallab et al.<sup>12</sup> detected more levels of MDA in aggressive periodontitis than in chronic periodontitis and healthy subjects. In our estimation of salivary MDA in healthy, moderate, and severe periodontitis, increased levels were seen with increased severity of disease. Our results are akin to Khalili and Biloklytska,<sup>13</sup> where a significant increase in the MDA level existed in the samples obtained from the three groups of patients (early, moderate, and severe periodontitis) compared with the control subjects, which incrementally elevated as a function of the progression in disease severity among the three groups of patients in comparison with the healthy control subjects. Having established the oxidative distress milieu of periodontal disease, we set forth to investigate the novel biomarker MHR ratio. Monocytes, during inflammation produce a multitude of cytokines, which will further aggravate the inflammatory process, whereas, HDL cholesterol, which has anti-inflammatory, antioxidant, and antithrombotic properties, strongly decreases pro-inflammatory and pro-oxidant effects of monocytes.<sup>14</sup> So, it seems convincing to incorporate both values into a single MHR ratio, which can echo the underlying inflammation and serve as an inflammatory as well as an oxidative stress marker. White blood cells represent the main constituent of the immune and inflammatory response, and increase in any one of the components of white blood cells reflects the inflammatory process. Many authors have shown that venous blood of patients with chronic periodontitis had elevated white blood cell levels.<sup>15,16</sup> In our study, the circulating levels of monocytes are higher in periodontitis group compared with the healthy individuals corroborating with the findings of Buhlin et al.<sup>17</sup> Moreover, systemic inflammation characterized by an elevated white blood cell count in venous blood could be caused by higher endotoxin levels detected in the plasma of periodontitis patients, compared with those in healthy patients.<sup>18</sup> Chronic inflammation can cause dyslipidemia, a common link connecting periodontal disease, cardiovascular disease, and diabetes mellitus. Literature survey yields conflicting results on the correlation between serum lipids and periodontal health. As early as 1999, Ebersole and Taubman<sup>19</sup> observed a transient decline in HDL in ligature-induced periodontitis in a nonhuman primate model. Fentoğlu et al.<sup>20</sup> noticed that a statistically significant negative correlation was observed between HDL and clinical attachment loss, and statistically significant positive correlations were observed between clinical attachment loss and triglycerides, total cholesterol, and low-level-density cholesterol. Interestingly, few studies have reported decreased levels of anti-atherogenic HDL in subjects with periodontitis.<sup>5-7</sup> In our study, serum HDL was reduced in chronic periodontitis patients, which was statistically significant when compared with controls. The results are on par with the above-mentioned subset of studies. As for MHR, elevated levels were noted in periodontitis compared with healthy subjects. Similar increased HDL monocyte ratio has been seen in cardiovascular disease, diabetes, smokers, and many more systemic diseases.<sup>21-23</sup> Inflammation is the major common development/progression factor in all these chronic diseases. This pilot study is the first, associating HDL monocyte ratio to periodontal disease. Moreover, in the present study, MHR was found significantly higher in moderate and severe periodontitis groups when compared with the control group and MHR increased as the severity of disease increased. Therefore, higher MHR ratio may be associated with adverse periodontal inflammatory processes and can serve as a useful marker of chronic inflammatory stress. Since the test is relatively easy, readily available, and cheap, it can be used

for follow-up of chronic periodontitis patients. Certain limitations of the study must be acknowledged. The biochemical parameters were assessed in serum, following the studies done in other systemic diseases, whereas gingival crevicular fluid would have been a more promising medium. Periodontal disease associated with other comorbidities like cardiovascular disease, diabetes, obesity, and environmental influences like smoking has not been included. An expanded study with a larger sample size, using gingival crevicular fluid as the sample with more specific severity categories of periodontitis, will shed more light on the clarity of MHR association with periodontal disease.

## CONCLUSION

This study was the first to investigate MHR in periodontitis subjects. The results indicated that periodontitis subjects had a higher MHR ratio than the healthy subjects. It is simple and cost-effective and serves as a beneficial marker of inflammation and oxidative stress of periodontal disease.

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