

Salivary Monocyte Chemoattractant Protein-1 Levels in Obese Young Adults: A Cross-sectional Study on Early Inflammatory Profiling

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Abstract

Background: Monocyte chemoattractant protein 1 (MCP-1) is a major inflammatory cytokine in obesity that increases the risk of various systemic diseases. This study explored the association between obesity and salivary MCP-1 levels in healthy young adults as a potential early indicator of inflammatory risk. **Methods:** This cross-sectional study was conducted at Sree Balaji Dental College and Hospital, Chennai, India, and involved 70 participants, aged between 18 and 25 years, grouped into two groups. Group I ($n = 35$) included obese adults and Group II ($n = 35$) included nonobese adults based on the body mass index (BMI). 3 mL of unstimulated saliva was collected and assessed for MCP-1 by ELISA. Data were analyzed using independent t-tests, and correlations between salivary MCP-1 levels and BMI were assessed with Pearson's correlation. $P \leq 0.05$ was considered statistically significant. **Results:** Salivary MCP-1 levels were significantly higher in the obese group (2.90 ± 0.74 pg/mL) compared to the nonobese group (1.94 ± 0.17 pg/mL). An independent samples *t*-test revealed a statistically significant difference between the groups ($P < 0.001$). The effect size, calculated using Cohen's *d*, was 1.79, indicating a very large magnitude of difference. Furthermore, there was a positive correlation between BMI values and salivary MCP-1 levels ($P < 0.04$), indicating adiposity as a risk for inflammation and associated complications. **Conclusion:** The results of the study show that salivary MCP-1 could be used as a noninvasive and early diagnostic marker for early inflammatory changes in obesity. Obese individuals showed significantly higher salivary MCP-1 levels, which correlated positively with BMI, indicating increased subclinical inflammation linked to adiposity.

Keywords: Biological markers, inflammation, monocyte chemoattractant protein-1, obesity, saliva

INTRODUCTION

Data were analyzed using independent t-tests, and correlations between salivary MCP-1 levels and BMI were assessed with Pearson's correlation. It is a prevalent public health problem across the globe.^[1] Approximately 39% of the global adult population were classified as overweight (body mass index [BMI] >25 kg/m²) or obese (BMI >29.9 kg/m²). The number of obese individuals has trebled since 1975.^[2] Nearly, 39% of the individuals over the age of 18 years are obese across the globe.^[2] India ranks third in obesity, with 64 million individuals being overweight or obese among the general population.^[3]

Obesity has also emerged as a global pandemic, with 2.8 million losing their lives annually due to obesity and associated complications.^[4] Individuals in the age range between 18 and

25 years are considered the most vulnerable group for obesity in developing countries owing to lifestyle modifications.^[5]

Inflammation is the connecting link between obesity and metabolic syndromes.^[6] Interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor (TNF) alpha are the commonly known inflammatory markers associated with

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obesity. Most of the inflammatory markers associated with obesity like CRP, IL-6, and TNF alpha are assessed invasively.

In obese individuals, adipocytes in addition to inflammatory mediators also secrete a major chemokine, monocyte chemoattractant protein-1 (MCP-1), also known as CC chemokine ligand-2.^[7] MCP-1 draws the monocytes out of circulation and convert them to macrophages in the tissue.^[8] Thereby, promoting chronic low grade inflammation, a hallmark of obesity and an established contributor to atherosclerosis and endothelial dysfunction.

MCP-1 also plays a major role in the development of diabetes, neuroinflammatory disease, and kidney and cardiovascular diseases by influencing multiple pathological mechanisms.^[9]

Subclinical inflammation associated with overweight and obesity may serve as an indicator of individuals at risk for obesity-related complications, given its central role in the development of insulin resistance and cardiometabolic disorders. Interestingly, not all obese individuals develop obesity-related inflammation and complications.^[10]

Considering the rise in obesity and associated metabolic syndrome and diabetes, the key approach to prevent obesity-associated complications would be early detection of inflammation. Furthermore, it is more important to identify inflammatory biomarkers associated with obesity and its correlation with BMI for early detection and prevention of obesity-associated complications.

A critical public health strategy for the primary prevention of obesity-related diseases is the early identification of individuals at elevated metabolic risk, distinguishing them from those considered “metabolically healthy obese,” who may not present with overt clinical symptoms despite excess adiposity. Elucidating the link between early-life obesity and chronic low-grade inflammation is critical for developing effective preventive interventions.^[11]

Understanding the existing knowledge gap, this study assessed salivary MCP-1 levels in clinically healthy young adults in comparison with obese adults to investigate its potential as a noninvasive biomarker for early detection of subclinical inflammation. The findings of this study support the utility of salivary MCP-1 in identifying low-grade inflammatory processes before the onset of clinical symptoms.

METHODS

Ethical considerations

This study was conducted in full accordance with the ethical principles outlined in the Indian Council of Medical Research and Declaration of Helsinki. The research protocol was reviewed and approved by the Institutional Ethics Committee of Sree Balaji Dental College and Hospital (SBDCH-IEC/23-01/03). This study was carried out in accordance with the strengthening the reporting of observational studies in epidemiology guidelines.

Type of sampling and reason for selection

This was a hospital-based cross-sectional study conducted at the Department of Oral Pathology and Microbiology at Centre for Oral Cancer Prevention Awareness and Research, Sree Balaji Dental College and Hospital, Chennai, Tamil Nadu, India, between June 2023 and July 2023. A convenience sampling method with purposive selection was employed.

Patient consent statement

Written informed consent was obtained from all the individuals participating in the study.

Inclusion criteria

A total of 70 subjects, between 18 and 25 years of age, who were systemically and orally healthy were recruited for the study. Based on the BMI guidelines given by the WHO, participants with a healthy BMI ranging from 18.5 to 24.9 kg/m² were categorized as nonobese and participants with BMI over 30 kg/m² were considered obese.^[2]

They study population were grouped into two groups as follows:

- Group I ($n = 35$) – Obese young adults
- Group II ($n = 35$) – Nonobese young adults.

Exclusion criteria

Participants using tobacco and tobacco-related products or alcohol and patients having any systemic diseases, thyroid disorders, inflammatory diseases, or taking anti-inflammatory drugs were excluded from the study.

Pregnant and lactating women and women taking oral contraceptives were also excluded. A thorough oral examination was done by a trained dentist, and subjects with oral lesions and other oral diseases were also excluded from the study.

Data availability statement

This article included all data generated or analyzed during the study. Further enquiries can be directed to the corresponding author.

Assessment of dietary patterns, physical activity, and socioeconomic background

Information on dietary habits, physical activity, and socioeconomic background was obtained through an interviewer-administered questionnaire. Participant responses were recorded by the researcher during face-to-face interaction, rather than through self-administered forms. Dietary habits were categorized as vegetarian, nonvegetarian, or mixed diet. Physical activity was assessed based on whether the participant engaged in any specific activity aimed at weight reduction. These primarily included walking, gym-based exercises, and home workouts of mild-to-moderate intensity as reported through self-administered questionnaires. Socioeconomic background was broadly classified, with all participants falling under the middle-income category.

Anthropometric measurements

The height of each participant was measured using a stadiometer, accurate to 0.1 cm, and subsequently converted

to meters for analysis. Body weight was obtained using a calibrated digital weighing scale with a precision of 0.1 kg, without shoes and with light clothing. BMI was then calculated using the standard formula: weight in kilograms divided by the square of height in meters (kg/m^2). Participants were classified as nonobese and obese based on the BMI values.

Sample size calculation

This study was exploratory in nature. Although a power analysis was not conducted prior to participant recruitment, a *post hoc* power calculation was performed using parameters for an independent samples *t*-test. With a total sample size of 70 participants (35 per group), the analysis indicated that the study had approximately 80% power to detect a moderate effect size (Cohen's $d = 0.5$) at a significance level of 0.05 (two-tailed). This suggested that the sample size was adequate to identify statistically meaningful differences between the groups.

Saliva collection

The unstimulated whole saliva was collected from the selected participants by spitting method. Approximately 3 mL of unstimulated saliva was collected in a sterile wide mouthed container and was processed on the same day. Saliva was collected from each patient between 9 AM and 11 AM while seated in upright position. Patients were asked to avoid eating, drinking, or gum chewing for 1 h before collection. Patients rinsed their mouth with water and then expectorated whole saliva into sterile tubes. The collected salivary samples were centrifuged at 2000 rpm for 10 min, and the supernatant was used for the study. The supernatant was then stored at -70°C until further analysis.

Monocyte chemoattractant protein assay

The levels of MCP-1 in saliva were estimated using an enzyme-linked immunoassay kit (Shanghai Coon Koon Biotech Co., Ltd, Shanghai, China). The assay was performed according to the manufacturer's instructions. The lower limit of detection was 1 pg/mL, and the assay showed high sensitivity and specificity for MCP-1.

The intra-assay coefficient of variation (CV%) was $<6\%$, and the inter-assay CV% was $<10\%$, indicating good precision and reproducibility. All samples were analyzed in duplicate to ensure accuracy.

A volume of 50 μL of the standard solution of concentration 20–320 pg/mL was added to the standard well. 40 μL of the salivary sample and 10 μL of anti-MCP1 antibody was added to a 96-well microELISA plate (dismountable). Then, 50 μL of streptavidin-HRP was added to sample wells and standard wells. After incubation at 37°C , 60 min, the unbound complex was removed from each well and washed with 350 μL wash buffer.

Then, 50 μL of chromogen A and chromogen B was added to each well and incubated in dark for 10 min at 37°C . To stop the reaction, 50 μL of stop solution was added to each well. The absorbance value of each well was measured at 450 nm.

All analyses were performed in duplicates. Standards were included on all runs, and all the results were reported within the linearity of the assays. The readings were made at 450 nm with a microplate spectrophotometer.

Statistical analysis

All the data were analyzed using SPSS Version 20 (IBM Chicago Inc., NY, USA). The results on continuous measurements were presented as mean \pm standard deviation and on categorical measurements were presented in number (%). Categorical data were analyzed for statistical significance using Chi-square test. All continuous data were subjected to Kolmogorov–Smirnov test for normality. Independent *t*-test was used to analyze the difference in the means of continuous variables. Pearson's correlation coefficient test was used to find the degree of correlation between BMI and MCP1 concentration. For all comparisons, $P < 0.05$ was considered statistically significant.

RESULTS

This study was designed as a cross-sectional study involving 70 young individuals. The total sample size of 70 in the present study was considered adequate since the power of the study calculated was $>80\%$ at the level of significance of 0.05.

The anthropometric details are represented in Table 1. As the study participants are age matched, the mean age did not show any statistical significance. Furthermore, the height of the individuals across the study group did not show any variance.

The majority of the participants in both the obese and nonobese groups reported following a mixed diet. Specifically, 97.2% ($n = 34$) of participants in each group consumed a combination of vegetarian and nonvegetarian foods, while only 1 (2.8%) participant in each group reported adhering to a strictly vegetarian diet. None of the participants identified as exclusively nonvegetarian. A Chi-square test comparing dietary pattern distribution between the groups showed no statistically significant difference ($P = 1.000$).

Table 1: Anthropometric characteristics of the study population

	Nonobese adults	Obese adults	P
Age (years)	20.30 \pm 2.45	21.40 \pm 2.34	0.08 (NS)
Height (cm)	161.40 \pm 10.2	161.07 \pm 14.3	0.09 (NS)
Weight (kg)	55.96 \pm 12.8	80.04 \pm 21.1	<0.001
BMI (kg/m^2)	21.83 \pm 1.47	28.54 \pm 2.71	<0.001
Weight loss activity (%)			
Engaged	20	22.9	0.69 (NS)
Not engaged	80	77.1	
Gender (%)			
Male	13.3	3.3	0.04*
Female	86.7	96.7	

* $P < 0.05$. Values are represented as mean \pm SD. NS: Not significant statistically, BMI: Body mass index, SD: Standard deviation

There was very high statistical significance with respect to weight as one study group was comprised of only obese individuals. Similarly, the BMI values were also found to be high in the obese individuals ($28.54 \pm 2.71 \text{ kg/m}^2$).

With respect to physical activity, a subset of participants in both the groups reported engaging in activities specifically aimed at weight loss. Among nonobese participants, 20% ($n = 7$) of participants reported such efforts, compared to 22.9% ($n = 8$) in the obese group. Conversely, 80% ($n = 28$) of the nonobese and 77.1% ($n = 27$) of the obese participants reported not engaging in any weight loss activity. The difference between the groups was not statistically significant (Chi-square test, $P = 0.69$).

The present study was not gender matched. There were a greater number of female participants than male participants across the study groups, and it showed a statistically significant difference ($P < 0.05$).

The salivary levels of MCP1 assessed by ELISA are represented in Table 2. The mean salivary levels of MCP1 between the study groups were analyzed by Student's *t*-test, and it showed a very high statistically significant difference. The MCP1 levels demonstrated in the obese individuals were much higher when compared to nonobese controls. The relationship between BMI and MCP-1 levels had a weak positive correlation ($r = 0.23$), and the relationship was found to be statistically significant.

DISCUSSION

The present study evaluated the level of salivary MCP 1 in young obese adults in comparison with nonobese adults. The primary research question focused on whether salivary MCP-1 levels differ significantly between obese and nonobese individuals. We have demonstrated higher levels of MCP1 in obese adults when compared with nonobese individuals [Figure 1]. There was also a weak positive correlation between MCP1 and BMI, indicating that with increase in BMI, there is a subsequent increase in the salivary levels of MCP1.

This study is age matched; therefore, we have eliminated the bias of age influencing obesity and inflammatory status of an individual. No significant variation in height was observed between the study groups.

Females constituted 96.7% of the obese group in the study. Gender skew in the study cohort reflected the demographic

	MCP (pg/mL)	P
Nonobese adults	1.94±0.17	<0.001
Obese adults	2.9±0.74	

Parameter	Chemokine MCP	
	Pearson correlation	P
BMI	0.23	0.04*

* $P < 0.05$. All values are represented as mean±SD. BMI: Body mass index, SD: Standard deviation, MCP: Monocyte chemoattractant protein

profile of the outpatient population attending the site during recruitment.

Due to the observational nature and sample constraints of the study, gender was not controlled during group allocation. Gender is always considered an important variable in obesity and our study also reflects the same.^[12,13] In line with this, a study by NIHANES has reported higher prevalence of obesity in women than men.^[12] Furthermore, gender influences the development of central or peripheral adiposity. Furthermore, the expression of inflammatory cytokine such as MCP1 is known to exhibit sexual dimorphism, probably due to the influence of estrogen that plays a significant anti-inflammatory role in attenuating meta inflammation.^[13,14] A significant increase in MCP1 levels was reported in females with metabolic syndrome in comparison with males of the similar age group.^[15]

Emerging evidence indicates that metabolic perturbations associated with obesogenic lifestyles, particularly high-fat dietary intake and physical inactivity, can upregulate proinflammatory chemokines such as MCP-1, thereby promoting chronic low-grade inflammation.^[16] On the other hand, anti-inflammatory diet was found to lower serum MCP1 levels in obese women.^[17] In this study, all participants reported consuming a mixed diet, incorporating both vegetarian and nonvegetarian food sources. While dietary composition plays a key role in modulating systemic and salivary inflammatory markers, the lack of variation in dietary patterns among participants may have minimized the differential impact of diet on MCP-1 expression.

With respect to physical exercise, this study found no statistically significant difference in self-reported engagement in weight loss-related activity between obese and nonobese participants, suggesting that both the groups exhibited comparable behavioral efforts toward physical activity.

MCP-1 has a clear positive association with percentage body fat only in the obese group.^[18] Waist hip circumference was

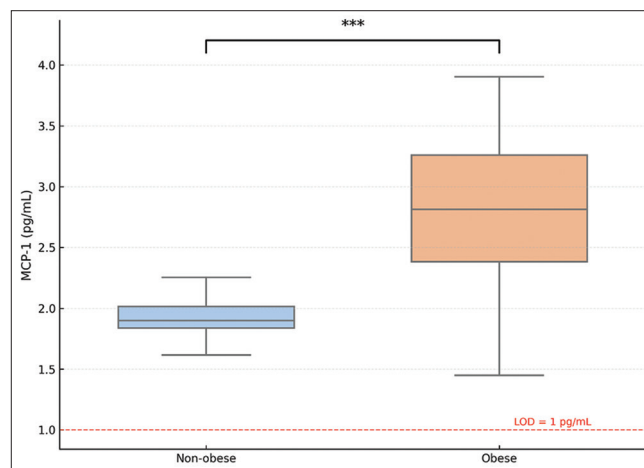


Figure 1: Comparison of salivary monocyte chemoattractant protein 1 (MCP-1) levels between obese and nonobese individuals. Box plots represent the distribution of MCP-1 concentrations in each group, with individual data points overlaid. *** $P < 0.001$

correlated increased serum MCP1 levels in obese children in Mexico.^[19] Increased serum MCP1 levels were found to be associated with dyslipidemia in Chinese population,^[20] and a proteomics-based study conducted in a European population has also supported these findings.^[21] The expression of MCP1 has been found to be more in individuals with greater visceral and abdominal obesity.^[22] In line with this, the present study also showed a positive correlation between BMI and salivary levels of MCP1.

Obesity is generally accompanied with low-grade inflammation. MCP1, a chemokine secreted by adipocytes, plays a significant role in inflammation. MCP1 gene polymorphism was found to be associated with inflammation and metabolic diseases such as type 2 diabetes in Korean population.^[23] Elevated circulating MCP-1 levels have been implicated in cardiovascular disorders and are recognized as a risk factor for stroke.^[24,25] Serum MCP1 was identified as a potential contributor to atherosclerosis, neurocognitive decline, and postoperative organ failure.^[26] A study by Li *et al.* has further reported MCP1 as a clinical prognostic marker for coronary artery disease.^[27]

In addition, MCP-1 plays a pivotal role in T-cell migration, angiogenesis, and osteoclastogenesis.^[28-30] It has also been implicated in oxidative stress pathways, highlighting its broader involvement in inflammation-driven conditions.^[31] Similarly, the role of MCP1 in cancer and tumor progression by binding to CCR2 receptor is also reported.^[32] MCP1 levels were also found to be aberrant in various infectious diseases.^[33] MCP1 is also considered a valuable biomarker of inflammation in nephropathy^[34] and sepsis.^[35] Elevated levels of MCP-1 have been observed in neurodegenerative conditions such as Parkinson's disease and Alzheimer's disease,^[36] as well as in rheumatoid arthritis.^[37] Salivary MCP1 levels were found to be increased in periodontitis and decreased in salivary gland tumors. Salivary MCP1 was increased in sleep disorders.^[38,39]

Recent studies suggest that salivary levels of cytokines, specifically MCP-1, may reflect systemic inflammatory states, highlighting their potential utility in early detection and public health surveillance. Given its pivotal role in inflammatory signaling and disease progression, MCP-1 is increasingly recognized as a promising biomarker for diagnosis and monitoring. Moreover, advancing our understanding of MCP-1 secretion dynamics and its receptor interactions is essential for elucidating its functional significance in both physiological and pathological states.

While there is a large body of evidence available to correlate the MCP1 levels with subclinical inflammation, existing studies are based on the assessment of MCP1 invasively, using blood samples, that may not be suitable for large-scale screening, especially in young or asymptomatic populations.

Saliva, on the other hand, offers a noninvasive, easily accessible, and cost-effective alternative for biomarker analysis. Recent studies suggest that salivary levels of cytokines, including MCP-1, may reflect systemic inflammatory states, highlighting

their potential utility in early detection and public health surveillance.

An important aspect when interpreting salivary inflammatory markers is the consideration of potential confounders such as dietary habits, physical activity, socioeconomic background, and oral health status. By incorporating the abovementioned factors in the present study, the impact of potential confounders on the study design is largely minimized. Moreover, only individuals with clinically healthy oral conditions were included in this study, thereby further eliminating the possibility of local oral inflammation influencing salivary marker levels.

The present study's methodological strength also lies in the careful selection of age matched, healthy, nonsmoking, nonalcohol-consuming individuals without any systemic disease or inflammatory conditions, reducing the influence of potential confounding factors.

Despite adjusting for known confounding variables, the observed weak correlation between salivary MCP-1 and BMI may be attributed to the multifactorial regulation of MCP-1 expression. Further longitudinal, gender-matched, multicentric studies involving larger cohorts are imperative to validate the potential of salivary MCP-1 as a noninvasive biomarker for the early detection of subclinical inflammation associated with obesity.

In light of emerging evidence implicating low-grade inflammation in obesity-related pathophysiology, the quantification of salivary MCP-1 in obese young adults represents a promising approach to elucidate subclinical inflammatory processes. This study addresses a critical gap in current knowledge by examining the association between adiposity and salivary MCP-1 concentrations, thereby contributing to the development of noninvasive biomarkers for early identification of individuals at elevated inflammatory status.

To summarize, our study proposes a strong relationship between salivary levels of MCP and obese young adults. This study also gives an insight for enabling MCP as a biomarker and diagnostic tool for detecting the risk of inflammation and associated diseases in obese young adults.

CONCLUSION

The primary prevention of obesity-related diseases is the early identification of individuals at elevated metabolic risk, distinguishing them from those considered "metabolically healthy" obese, who may not present with overt clinical symptoms despite excess adiposity.

There is an increase in the prevalence of obesity and overweight due to the urbanization and lifestyle changes including diet and lack of physical activities.

Young adults between the ages of 18 and 25 years are in a period of "transition" from adolescence to adulthood. The significant results of the present study with salivary

MCP-1 could help healthcare professionals assess the risk of inflammation and inflammation-mediated systemic diseases through a less invasive method even at the dental setup at an early stage in obese individuals.

Saliva-based inflammatory markers may expand the repertoire of biomarkers available for quantifying subclinical inflammation in individuals with obesity.

Outcome of the study

The results of this provide preliminary evidence supporting the use of salivary MCP-1 as a noninvasive biomarker for early detection of inflammation associated with obesity.

Rationale of the study

Young adults are in a critical transition phase where early risk factors for systemic diseases often manifest. Salivary MCP-1 assessment offers a less invasive tool to identify inflammation in obese individuals, even in routine dental settings.

Limitations of the study

The present study is limited by its relatively small sample size and single-center design. In addition, the cross-sectional nature of the study does not allow for the assessment of long-term outcomes or causality.

Author contribution

Dr. Mathangi. R: Conceptualization, methodology, investigation, writing original draft; Aravindha Babu-Conceptualization, Writing, review and editing; Dr.Anusha &Dr. E. Rajesh – Methodology and data collection.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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