Association between nonalcoholic fatty liver disease and inflammatory periodontal disease: A case-control study

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Abstract:

Background: Recent evidence suggests an interconnection between chronic periodontal disease and systemic diseases. Aim: The aim of this study is to evaluate the possible association between nonalcoholic fatty liver disease (NAFLD) and inflammatory periodontal disease among north Indian population. Settings and Design: Tertiary health care center, cross-sectional case-control observational study. Materials and Methods: A total of 40 cases, i.e., patients with NAFLD and 40 healthy volunteers were included over a period of 8 months and their periodontal status was compared. The status of their hepatic health was ascertained by anthropometric, imaging, and biochemical evaluation including ultrasound examination of abdomen and transient elastography. Statistical Data Analysis: Paired t-test, multivariate logistic regression analysis using IBM SPSS STATISTICS (version 22.0, Armonk, NY: IBM Corp). Results: The study revealed that only 11.9% and 20% of participants had periodontitis, in healthy controls and hepatic disease patients, respectively. A statistically significant difference was observed in clinical parameters of periodontal status, except for malocclusion. Comparative analysis of tumor necrosis factor-α (TNF-α), interleukin-6, C-reactive protein, and cytokeratin-18 revealed differences in mean scores, though statistically nonsignificant. Only aspartate transaminase, number of missing teeth, and bleeding on probing (BOP) were observed with higher odds ratios for hepatic disease patients. Spearman correlation analysis revealed significant positive correlations between TNF-α and BOP, for cases. Conclusion: Patients with hepatic disease showed a higher prevalence of periodontal disease, worse oral hygiene and periodontal health status compared to healthy individuals.

Key words: Bleeding on probing, case control, nonalcoholic fatty liver disease, oral health, periodontal disease, systemic health, tumor necrosis factor

INTRODUCTION

Periodontal disease is an immune inflammatory disease caused by microbial infection and results in the loss of gingiva and of the supporting structures of the teeth.[1-8] The shedding of lipopolysaccharide from periodontal flora stimulates the endothelial cells, monocytes, and macrophages to initiate a proinflammatory response resulting in long-standing sustained increase in many cytokines enhancing inflammation, i.e., interleukins 1β (IL-1β), 6, and tumor necrosis factor (TNF)-α.[3,4,6-9,11] A huge body of contemporary epidemiological studies have reported periodontal disease as a risk factor for various overall health conditions, including cardiovascular disease, type 2 diabetes, adverse pregnancy outcomes, and rheumatoid arthritis.[3,24] The term nonalcoholic fatty liver disease (NAFLD) encompasses a disease spectrum which includes variable degrees of simple steatosis (nonalcoholic fatty liver disease, NAFLD), nonalcoholic steatohepatitis (NASH) and cirrhosis.[13] The overall prevalence of NAFLD varies from 15% to 40% and 9%-40% in Western countries and in Asian countries, respectively.[14-16] NAFLD is an emerging cause of liver disease in our country as well. The prevalence of NAFLD is 9%-32% in the general Indian population, with a higher incidence among specific groups, namely, obese and diabetic patients.[17-23]
With the rising incidence and prevalence, an enhanced understanding of the diverse aspects of NAFLD is appearing and is alarming in context of its clinical implications,\cite{26,27} Approximately 10%–30% have the potentially advancing type of NAFLD, NASH (NASH/hepatic cellular injury and inflammation). Further, 25%–40% of NASH patients may progress to liver fibrosis over time, resulting in cirrhosis in 20%–30%. Such cirrhotic patients are highly susceptible to develop hepatocellular carcinoma (2.6%/year).\cite{28} NASH is considered synonymous to the metabolic syndrome (MS) affecting the liver, i.e., as an associate morbidity to diabetes mellitus (DM) type 2, hyperlipidemia, and hypertension (HTN).\cite{29}

NAFLD has been associated with infections of gastrointestinal tract, such as small intestinal bacterial overgrowth.\cite{30} Oral bacteria have also been implicated in the induction of endotoxemia and subsequent hepatic inflammatory responses directly or indirectly through imparting alterations in gut flora.\cite{31} More recently, Porphyromonas gingivalis, most prevalent pathogen was detected in significantly higher frequency in periodontal disease in NAFLD patients than in the non-NAFLD participants (46.7% vs. 21.7%, odds ratio [OR]: 3.16). Moreover, NASH patients showed a higher detection rate of P. gingivalis than that in the non-NAFLD participants (52.0%, OR: 3.91).\cite{32}

Indian NAFLD patients vary from their Western counterparts in diverse aspects as these are less obese and have lower frequency of DM and MS.\cite{33} As available literature regarding the interrelationship among NAFLD, periodontal disease and MS is largely from non-Indian populations. The current investigation was planned to conduct a cross-sectional case-control observational study aimed at investigating the possible association between NAFLD and periodontitis in regional population.

**SUBJECT POPULATION AND CATEGORIZATION**

Forty patients diagnosed as suffering from NAFLD in the Department of Hepatology, PGIMER, Chandigarh, a tertiary care Centre were included over a period of 6 months (case group) and their periodontal status were compared with 40 normal healthy volunteers (HVs) (control group) selected from the accompanying attendants other than those selected for this study. The study protocol was duly reviewed and approved by the Institutional Ethics Committees of both institutes (Ref no’s: PUIEC/2016/18/1-A/20/05 and PGI/IEC/2016/349). All study participants were explained about the need and detailed course of the study and were asked for the voluntary participation in the investigation. An informed consent regarding willingness to participate in study was obtained from the patients in written and signed format.

**Inclusion criteria**

**The nonalcoholic fatty liver disease group (cases)**

Nonalcoholic fatty liver disease patients

- Showing hepatic steatosis on liver ultrasound and at least 1.5 times increased levels of liver enzymes, i.e., aspartate transaminase (AST) and alanine transaminase (ALT) at least for a duration of >3 months, and where available, liver biopsy confirmed diagnosis of NAFLD

**Exclusion criteria**

- Absence of alcohol intake history or intake limited to <20 g/day
- Absence of viral markers (HBsAg and anti-hepatitis C virus), autoimmune markers (antinuclear anti bodies, anti-smooth muscle antibody), anti-liver kidney-microsomal antibody anti-mitochondrial antibody), Kayser–Fleischer rings
- Normal ceruloplasmin levels and iron studies of the patients.

**Healthy volunteers (controls)**

This group included healthy controls without a history of DM and with normal blood pressure, abdominal ultrasound, liver enzyme levels, and fasting plasma glucose.

**Study method**

**Anthropometric, imaging, and biochemical evaluation**

All patients with NAFLD underwent a detailed evaluation in the Department of Hepatology of a tertiary care medical center. The evaluation included a detailed medical history keeping inclusion and exclusion criteria in mind. Family history of diabetes, HTN, coronary artery disease, and stroke was also recorded. Body mass index (BMI) and waist-hip ratio for central obesity and abdominal examination for any organomegaly were recorded. After routine hematological, biochemical (including fasting plasma glucose, lipid profile), all patients were subjected to an ultrasound examination of abdomen and transient elastography (fibroscan) for the assessment of hepatic steatosis and fibrosis.\cite{34}

HVs were also assessed in the Department of Hepatology and underwent selective investigations of liver function tests, fasting plasma glucose, and abdominal ultrasound.

**Assessment of severity of hepatic disease**

- Hepatic steatosis was diagnosed on abdominal ultrasonography and was graded into mild, moderate, and severe as per Saverymuttu et al.\cite{35} In addition to ultrasound, hepatic steatosis was also graded noninvasively into S0, S1, and S3 based on the controlled attenuation parameter (CAP)-a software available with the transient elastography (fibroscan) machine\cite{36}
- Hepatic inflammation – patients with NAFLD were assessed with the help of serum biomarkers of hepatic inflammation and apoptosis. Noninvasive biomarkers included TNF-alpha, IL-6, C-reactive protein (CRP), and (CK)-18\cite{37}
- Hepatic fibrosis – was assessed noninvasively by measuring the liver stiffness measurement (LSM) with the help of transient elastography. Patients were divided into NASH and no-NASH based on the LSM (LSM ≥7 Kpa = NASH). In addition to Fibroscan, and histological assessment was made according to NAFLD activity score in patients who underwent liver biopsy\cite{38} and patients were divided into NASH (absent), NASH (borderline), and NASH.
Periodontal examination in patients with nonalcoholic fatty liver disease and healthy volunteers

A single previously calibrated and trained examiner made all measurements for parameters of periodontal status in the Department of Periodontology, Dr. HS Judge Institute of Dental Sciences, Punjab University, Chandigarh, India. The examiner was blinded about the hepatic status of patients. Probing pocket depths (PDs) were measured at standard six sites per tooth using a calibrated periodontal probe. Gingival recession was recorded as the distance from the cementoenamel junction to the gingival margin. Clinical attachment loss (CAL) and the percentage of bleeding sites in each participant were also noted.

Gingivitis was categorized as the absence of PD (≤3 mm), bleeding on probing (BOP), and clinical signs of gingival inflammation. Chronic periodontitis was defined by the presence of two or more sites with PD (≥4 mm), attachment loss (≥4 mm), and BOP. Periodontal disease was ascertained by radiographic alveolar bone loss. Chronic periodontitis was further classified into categories based on the severity of involvement: incipient (4−5 mm CAL) and moderate/severe (≥6 mm CAL). Periodontal health was determined when there was an absence of obvious clinical inflammation, BOP, and no periodontal attachment loss or bone loss.[37]

Table 1a: Demographic and hepatic status parameters in NAFLD and HVs

| Parameters | Study Group | HVs |
|------------|-------------|-----|
| Age (y)    | 44±10.90    | 30.24±7.30 |
| Sex(Male: Female) | 14:26 | 14:28 |
| AST (U/L)  | 73.10±88.26 | 23.36±8.85 |
| ALT (U/L)  | 70.25±38.18 | 25.09±15.11 |
| TGs (mg/dL) | 190±80.8 | - |
| HDL (mg/dL) | 41.3±7.4 | - |
| Fibro scan | - | - |
| LSM (Kpa)  | 5.96±1.94 | 306.35±56.05 |
| CAP        | - | - |

NAFLD: Non-alcoholic Fatty Liver Disease, HV: Healthy Volunteer, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, TGs: Triglycerides, HDL: high density lipoproteins, LSM: Liver stiffness measurement, CAP: Controlled Attenuation Parameter

Table 1b: Anthropologic and metabolic status parameters in NAFLD patients

| Parameters | Study Group |
|------------|-------------|
| Anthropometry |               |
| Normal BMI (BMI >18 TO <23) | 5 (12.5%) |
| Overweight (>23 to <25) | 11 (27.5%) |
| Class I Obese (>25 to <30) | 18 (45%) |
| Class II Obese (>30) | 6 (15%) |
| Metabolic syndrome |               |
| 0 Parameter | 0 |
| 1 Parameter | 1 (2.5%) |
| 2 Parameters | 14 (35%) |
| 3 Parameters | 16 (40%) |
| 4 Parameters | 4 (10%) |
| 5 Parameters | 5 (12.5%) |
| Metabolic Syndrome (>3 components) | 25 (62.5%) |
| Diabetes Mellitus (DM) | 10 (25%) |
| Hypertension (HTN) | 12 (30%) |

NAFLD: Non-alcoholic Fatty Liver Disease, BMI: Body Mass Index

Data analysis

The baseline demographic, anthropologic, and clinical data have been tabulated [Table 1 and Figures 1, 2]. Bilirubin, AST, ALT, debrid index simplified (DIS), calculus index simplified, missing teeth, malocclusion, number of sites, BOP, mean PD, mean CAL, PD, oral hygiene index simplified (OHIS) were expressed by mean ranks and standard deviations, and paired t-test was performed [Tables 2 and 3]. The prevalence of periodontitis based on the predefined CAL criteria have been presented by frequency distribution and compared between cases and controls [Table 4]. The periodontitis prevalence was further analyzed and compared within NASH and non-NASH patients in case group [Table 5].

Multivariate logistic regression models were applied and the association between periodontitis and risk indicators was expressed by ORs at 95% confidence interval [Table 6]. A spearman correlation analysis in hepatic status and oral health status parameters has been performed [Figure 3].

RESULTS

Forty NAFLD patients (14 males and 26 females) with a mean age 44 ± 10.90 years were included over an 8-month period. Five patients of the case subject population (12.5%) had normal body weight, 11 (27.5%) were overweight, 18 (45%) had class I obesity, and 6 (15%) had class II obesity [Table 1 and Figure 1]. Case population was analyzed for the presence of salient parameters of MS. 25 NAFLD cases (62.5%) had >3 components of MS [Figure 2]. A control group of 42 HV consisting of 14 males and 28 females with a mean age of 30.24 ± 7.30 were included during the same time span [Table 1]. All enrolled HVs were normotensive, nondiabetic, normal range cholesterol levels and normal level of hepatic enzymes AST and ALT. A comparative analysis of all hepatic health indicators was performed and significant differences were observed in liver enzymes (AST, ALT), bilirubin levels [Table 2]. Case controls were divided into NASH (10) and non-NASH (28) based on the LSM (LSM ≥7 Kpa = NASH). The mean scores for LSM were 8.8 ± 0.99 and 5.01 ± 1.05 for NASH and non-NASH patients, respectively (data not shown).

Comparison of hepatic health parameters in nonalcoholic steatohepatitis and non-nonalcoholic steatohepatitis

The mean CAP scores were 351 ± 33.1 and 291 ± 54.4 for NASH and non-NASH patients, respectively. The mean levels of all systemic biomarkers, namely, TNF-α, IL-6, CRP, CK18 were lower in non-NASH patients then observed in NASH patients. Inflammatory biomarkers, namely, TNF-α, IL-6, CRP, and CK18 revealed differences in mean scores, but no statistically significant differences could be traced in. A statistically significant difference was revealed in median scores of CAP, AST levels for NASH and non-NASH patients [Table 3].

Prevalence of periodontitis in nonalcoholic fatty liver disease and nonalcoholic steatohepatitis cases population

Only 11.9% and 20% of the study participants had periodontitis, with in the healthy controls and patients suffering from hepatic disease, respectively, in total study population [Table 4]. The prevalence of periodontitis was observed to be 17.2% and 30%, respectively, in non-NASH and NASH patients [Table 5].
Comparison of clinical parameters of periodontal health in nonalcoholic fatty liver disease and healthy volunteers

Table 3 shows the mean rank scores of clinical parameters of hepatic health as well as periodontal status for total study population. Statistically significant difference was observed in clinical parameters of periodontal disease, except for malocclusion between HVs and hepatic disease patients (cases) [Table 2].

Comparison of clinical parameters of periodontal health in nonalcoholic steatohepatitis and non-nonalcoholic steatohepatitis case population

Similarly, a statistically significant difference for mean rank scores of AST, interquartile range (IQR) and CAP scores was observed, when patients with NASH and non-NASH status were compared for hepatic health parameters. None of the periodontal health parameters

Table 2: Comparison of hepatic health parameters and oral clinical parameters in nonalcoholic fatty liver disease and healthy volunteers

| Clinical measurements | Controls Mean rank | Case NAFLD Mean rank | P (paired t-test) |
|-----------------------|-------------------|----------------------|-----------------|
| Bilirubin             | 32.98 (n=42)      | 49.64 (n=39)         | 0.001           |
| AST                   | 25.95 (n=42)      | 57.21 (n=39)         | 0.000           |
| ALT                   | 25.81 (n=42)      | 57.36 (n=39)         | 0.000           |
| DIS                   | 50.65 (n=42)      | 31.89 (n=40)         | 0.000           |
| CIS                   | 50.61 (n=42)      | 31.94 (n=40)         | 0.000           |
| Missing teeth         | 35.07 (n=42)      | 48.25 (n=40)         | 0.004           |
| Malocclusion          | 41.57 (n=42)      | 41.43 (n=40)         | 0.974           |
| BOP                   | 31.00 (n=42)      | 52.53 (n=40)         | 0.000           |
| Mean PD               | 46.14 (n=42)      | 36.63 (n=40)         | 0.052           |
| Mean CAL              | 31.45 (n=42)      | 52.05 (n=40)         | 0.000           |
| OHIS                  | 51.07 (n=42)      | 30.68 (n=40)         | 0.000           |

The P value is considered significant, for P<0.05. NAFLD – Nonalcoholic fatty liver disease; AST – Aspartate aminotransferase; ALT – Alanine aminotransferase; DIS – Debris index simplified; CIS – Calculus index simplified; BOP – Bleeding on probing; PD – Pocket depth; CAL – Clinical attachment loss; OHIS – Oral hygiene index simplified; P – P-value or probability value

Table 3: Comparison of hepatic health parameters and oral clinical parameters in non-alcoholic steatohepatitis and nonalcoholic steatohepatitis groups

| Clinical measurements | Controls Mean rank | Case NASH Mean rank | P (paired t-test) |
|-----------------------|-------------------|---------------------|-----------------|
| Bilirubin             | 17.79 (n=28)      | 24.30 (n=10)        | 0.111           |
| AST                   | 17.09 (n=28)      | 26.25 (n=10)        | 0.025           |
| ALT                   | 18.23 (n=28)      | 23.05 (n=10)        | 0.239           |
| SR                    | 19.83 (n=29)      | 20.50 (n=10)        | 0.834           |
| IQR                   | 17.50 (n=29)      | 27.25 (n=10)        | 0.019           |
| CAP                   | 16.74 (n=29)      | 29.45 (n=10)        | 0.002           |
| TNF-α                 | 18.52 (n=29)      | 24.30 (n=10)        | 0.166           |
| IL6                   | 19.64 (n=29)      | 21.05 (n=10)        | 0.735           |
| CRP                   | 18.79 (n=29)      | 23.50 (n=10)        | 0.260           |
| CK18                  | 20.84 (n=29)      | 17.55 (n=10)        | 0.431           |
| DIS                   | 18.69 (n=29)      | 23.80 (n=10)        | 0.214           |
| CIS                   | 19.47 (n=29)      | 21.55 (n=10)        | 0.614           |
| Missing teeth         | 20.48 (n=29)      | 18.60 (n=10)        | 0.631           |
| Mean PD               | 18.93 (n=29)      | 23.10 (n=10)        | 0.288           |
| Mean CAL              | 19.50 (n=29)      | 21.45 (n=10)        | 0.631           |
| BOP                   | 18.67 (n=29)      | 23.85 (n=10)        | 0.202           |
| Pocket depth          | 18.93 (n=29)      | 23.10 (n=10)        | 0.288           |
| OHIS                  | 19.00 (n=29)      | 22.90 (n=10)        | 0.349           |

The P value is considered significant, for P<0.05. NASH – Nonalcoholic steatohepatitis; AST – Aspartate aminotransferase; ALT – Alanine aminotransferase; SR – Success rate; IQR – Interquartile range; CAP – Controlled attenuation parameter; TNF-α – Tumor necrosis factor-alpha; IL6 – Interleukin-6; CRP – C-reactive protein; CK18 – Cytokeratin-18; PD – Pocket depth; CAL – Clinical attachment loss; BOP – Bleeding on probing; OHIS – Oral hygiene index simplified; P – P-value or probability value.
showed any statistically significant difference with in these groups [Table 3].

**Association between hepatic disease and clinical parameters of periodontal and hepatic health**

Multivariate logistic regression analysis was performed and the association between hepatic disease and study parameters was expressed as ORs. Only three study parameters viz. AST, number of missing teeth and BOP were observed with ORs with statistically significant difference for healthy and hepatic disease subjects [Table 6]. None of the indicator of periodontal status could be ascertained for differentiation of NASH and non-NASH patients based on logistic regression (results not shown).

**Co-relation of systemic biomarkers and clinical parameters of periodontal status**

Spearman correlation analysis revealed significant positive correlations between TNF-α and BOP, for case population group, i.e., patients with hepatic disease [Figure 3], whereas significant positive correlations were observed between BOP and CAL, PD and OHIS in all study population. (Data not shown).

**DISCUSSION**

The present study is the first report on the prevalence of periodontitis in NAFLD patients in the Indian population and explores the effect of periodontitis on the severity of NAFLD. The study findings regarding mean BMI and metabolic derangements are in agreement with the previous investigations conducted in India in NAFLD patient populations,[10,21] however these are lower than those reported from the West.[22-24] Findings revealed a periodontitis prevalence of 20% in patients of hepatic disease in total and 30% in patients suffering from NASH. The mentioned prevalence is definitely greater than the respective compared groups i.e., healthy controls (11.9%) and non-NASH individuals (17.2%), but the study could not delineate a statistically significant association between periodontal disease and NAFLD, owing to limited set of data.

There was a statistically significant difference for liver enzymes (AST, ALT), bilirubin levels and all clinical parameters of periodontal status evaluated, except for malocclusion between HVs and patients with hepatic disease, which surely points to some bearing of periodontal status on NAFLD status of the individual. Research studies in Japanese university students have reported significantly higher levels of alanine aminotransferase (ALT) in periodontitis patients.[30] The incidence of periodontal disease in healthy Japanese females was significantly greater when elevated serum levels of aspartate aminotransferase (AST), ALT and cholinesterase were present.[39,40] In a recent investigation of 7.7 years follow-up, 605 incident NAFLD cases were documented. In participants with CAL <3 mm, incidence of liver disease was slightly higher with <30% of sites affected and moderately higher in participants with ≥30% of sites affected (multivariable-adjusted incidence rate ratio = 1.28, 95% confidence interval [CI], 0.84, 1.95 and 1.60, 95% CI, 1.05–2.43), respectively. Although PD did not reveal a similar dose-response curve.[41]

When hepatic status and periodontal status parameters were compared between NASH and non-NASH patients, a significant difference was noted only in the AST levels, IQR and CAP scores. None of the periodontal status parameters or biochemical markers differed significantly, though NASH patients had worse scores than non-NASH patients for all indicators of periodontal health, except missing teeth. Inflammatory biomarkers also showed a trend toward higher levels in NASH as compared to non-NASH patients, with only CK-18 showing a reverse trend. A recent large sample investigation reported an association of periodontitis with hepatic steatosis and significant liver fibrosis (NHANES III cohort). Similar findings have been revealed regarding significant liver fibrosis in a prospective study of largely biopsy-proven NAFLD. In fact, authors confirmed the association with steatosis and demonstrated a gradient of periodontitis with worsening liver injury.[42] This investigation did not take in account of any biochemical markers such as hepatic enzymes or inflammatory serum markers, but similar clinical periodontal findings could not be replicated in our investigation.

AST, number of missing teeth and BOP could discretely differentiate between hepatic health and disease status, based on ORs calculated by multivariate logistic regression. A recent large study population revealed similar observation trends replicated for all periodontal parameters, i.e., adults

**Table 4: Prevalence of periodontitis (cases & controls)**

| No. of sites with CAL > 4mm * group Cross tabulation |  |
|-----------------------------------------------------|--|
| **Group**                                           | **NAFLD patients** | **Control** | **Total** |
| NO % within group                                   | 80.0%              | 88.1%       | 84.1%     |
| YES % within group                                  | 20.0%              | 11.9%       | 15.9%     |

NAFLD: Non-alcoholic Fatty Liver Disease, CAL: Clinical attachment loss

**Table 5: Prevalence of periodontitis (nash and non‑nash patients in cases)**

| No. of sites with CAL > 4mm * NASH (based on LSM >=7) * group Cross tabulation |  |
|-------------------------------------------------------------------------------|--|
| **Group** | **No. of sites with CAL > 4mm** | **NASH (based on LSM >=7)** | **Total** |
| NAFLD patients | NO % within NASH (based on LSM >=7) | 82.8% | 70.0% | 79.5% |
| YES % within NASH (based on LSM >=7) | 17.2% | 30.0% | 20.5% |

NAFLD: Non-alcoholic Fatty Liver Disease, NASH: Non-Alcoholic Steatohepatitis, LSM: Liver stiffness measurement, CAL: Clinical attachment loss
with steatosis showed a higher percentage of oral sites with BOP, periodontal probing depth ≥4 mm or CAL ≥3 mm. A statistically significant crude OR for steatosis was observed for all markers of periodontitis.\(^*\)

However, in the current investigation, some of the periodontal status parameters did reveal significant association with hepatic status indicators and provided significant insights into association. A significant positive correlation was observed between BOP and serum TNF-\(\alpha\) in patients suffering from hepatic disease. TNF-\(\alpha\) is an important inflammatory mediator secreted from hepatocytes and is known to perpetuate inflammation, proliferation and apoptosis in chronic liver disease.\(^{13,34}\) This raises the possibility of this mediator being a connecting link between periodontal and hepatic disease, as it has been previously implicated in many systemic diseases and conditions linked to periodontal disease.\(^{45-49}\) Other correlations observed in the study are also much in accordance with previously observed correlations which have been validated with significant body of evidence for example, LSM and CAP scores.\(^{40,52}\) IL-6 and CRP in patients with hepatic disease.\(^{50,51}\) Significant positive correlations were observed between BOP and CAL, PD and OHIS in all study population which is again a very old and time tested correlation in multiple cross sectional and longitudinal studies in periodontal disease patients.\(^{33}\)

There is mounting evidence documenting association between oral and systemic health, however the potential link between periodontitis and NAFLD patients has received scanty attention by now. Few previously published studies have provided conflicting results regarding the association of periodontal disease and NAFLD. Lack of infrastructure in terms of bringing in the hepatological and dental assessment together in a research environment is the major barrier perceived for meager number of such studies. The limited subject population reflects on the same aspects as we did this investigation in collaboration of a tertiary care medical center over a span of 1 year.

Another major challenge in studying associations between oral and systemic health is that diverse definitions of periodontitis, which pose serious differences in the measurement of disease in different studies, questioning the interpretation. Majority of investigations have been placed on diverse population of different ethnicity and variable experimental study settings and design. Hence, there is huge need of well-designed multicentric studies on this specific patient population, i.e., NAFLD patients with chronic periodontal disease to conclusively understand the association as well as causality link between periodontal disease and NAFLD.

**CONCLUSION**

Patients with hepatic disease showed a higher prevalence of periodontal disease as compared to healthy individuals. The same pattern was observed with respect to hepatic disease severity, revealing a higher prevalence of periodontal disease in patients of more severe hepatic disease. A worse oral hygiene and periodontal health status was observed in patients with hepatic disease compared to healthy individuals. Similar differences between NASH and non-NASH individuals, could not reach the statistical significance owing to the limited study population. Some of the systemic inflammatory biomarkers correlated well with clinical indicators of periodontal status, for example, TNF-\(\alpha\) and BOP for case population group, i.e., patients with hepatic disease pointing to the plausible mediating link of periodontal and systemic health. Thus, based on the current observations and within the limitations of investigation, it may be concluded, there appears to be an association between NAFLD and inflammatory periodontal disease, which needs to be ascertained by very well designed, multicentric cross sectional and longitudinal investigations.

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**Conflicts of interest**

There are no conflicts of interest.

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