Development of Non-alcoholic Fatty Liver Disease (NAFLD) in Young Obese Tribal Subjects of Tripura: Link between Low 25 (OH) Vitamin-D Levels and Immune Modulators

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Abstract

Background: There have been many studies conducted so far on Non Alcoholic Fatty Liver Disease (NAFLD) with its many aspects including its association with 25 hydroxy Vitamin D levels and its rather complex interplay with pro-inflammatory cytokines such as Interleukin-1a (IL–1a), Interleukin-6 (IL-6) and Tumour Necrosis Factor-Alpha (TNF-α), Interleukin-17a (IL–17a) and anti-inflammatory cytokines such as Interleukin-4 (IL-4) and Interleukin-10 (IL-10). This study was designed to show the development of NAFLD in the young tribal population of Tripura and the link between 25(OH) Vitamin D and pro-inflammatory cytokines (IL–1a, IL-6, IL–17a and TNF-α) and -inflammatory cytokines such as IL – 4 and IL - 10 and the development of NAFLD while at the same time throws light on the prevalence of 25(OH) Vitamin D deficiencies and the levels of pro-inflammatory cytokines in the study group.

Methods: The study is an analytical cross-sectional study with final population of 94 cases between 18 to 40 years of age fulfilling inclusion and exclusion criteria and an equal number of subjects from same tribal community age and sex matched taken as control population.

Results: There was a significant relationship between level of 25(OH) Vitamin D and fatty liver (OR: 9.46, 95% CI: 4.82 – 18.59; p < 0.001). The mean serum 25(OH) Vitamin D level in the cases was significantly higher than the controls (17.21 ng/ ml + 6.34 ng/ml vs 26.56 ng/ml + 10.63 ng/ml; p < 0.001). There was a significant difference between the mean serum levels of IL-1a (11.50 Pg/ml ± 2.75 Pg/ml vs 8.28 Pg/ml ± 2.08 Pg/ml; p < 0.001), IL-4 (0.69 Pg/ml ± 0.43 Pg/ml vs 0.84 Pg/ml ± 0.36 Pg/ml; p = 0.009), IL-6 (2.99 ± 1.11 Pg/ml vs 2.22 ± 1.08 Pg/ml; p < 0.001), IL-10 (6.50 ± 2.76 Pg/ml vs 5.23 Pg/ml ± 2.67 Pg/ml; p = 0.002), IL-17a (5.33 Pg/ml ± 2.22 Pg/ml vs 3.64 Pg/ml ± 1.99 Pg/ml; p < 0.001) and TNF-α (6.99 ± 2.81 Pg/ml vs 5.40 ± 3.08 Pg/ml; p < 0.001) of the cases and the controls Low serum 25(OH) vitamin D is capable to reduce FFA-induced insulin resistance both in peripheral tissues and in hepatocytes. Therefore, low serum vitamin D may predispose to intrahepatic lipid accumulation leading to NAFLD. A strong epidemiological overlap exists between NAFLD and hypovitaminosis D prevalence, as both conditions are widely spread among obese dysmetabolic individuals. Evidence suggests that endotoxin-mediated cytokines are important mediators of hepatic steatohepatitis. Cytokines are classified as T helper 1 (Th1) and T helper 2 (Th2) subtypes. The main proinflammatory Th1 cytokines are tumor necrosis factor-alpha (TNF-a), interleukin (IL)-1, IL-6 and interferon; whereas the main Th2 anti-inflammatory cytokines are IL-4 and IL-10. In general, Th1 cytokines induce Th1 cytokines and inhibit Th2 cytokine production and vice versa. Normally, there is a balance between proinflammatory and anti-inflammatory cytokines. IL-6 though initially considered as a hepatoprotector in liver steatosis, capable of reducing oxidative stress and preventing mitochondrial dysfunction, was however was found to be a key element in the acute phase response, mediating the synthesis of several acute phase proteins. It has been seen that not only the cytokine itself but also its soluble receptor is significantly increased in patients with NASH.

Conclusions: 25(OH) Vitamin D concentration are lower while that of IL–1a, IL-4, IL-6, IL-10, IL-17a and TNF-α are higher in subjects with fatty liver in comparison to those without. 25(OH) Vitamin D deficiency and high levels of serum IL-1a were independently associated with the risk of development of NAFLD.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a disorder that is characterized by a group of histological abnormalities identified on liver biopsy similar to those seen in alcoholic liver disease but occurring in patients who consume little or no alcohol. It has a spectrum that ranges from simple fatty liver (steatosis) to non-alcoholic steato-hepatitis (NASH) and NAFLD associated cirrhosis. Worldwide prevalence reports of NAFLD vary widely between 2.8 % to 46.0 %. Laboratory and clinical evidence supports the fact that peripheral insulin resistance and hyperinsulinemia are associated with NAFLD, even in lean patients without obvious glucose intolerance. Vitamin D is capable to reduce FFA-induced insulin resistance both in peripheral tissues and in hepatocytes. Therefore, low serum vitamin D may predispose to intrahepatic lipid accumulation leading to NAFLD. A strong epidemiological overlap exists between NAFLD and hypovitaminosis D prevalence, as both conditions are widely spread among obese dysmetabolic individuals. Evidence suggests that endotoxin-mediated cytokines are important mediators of hepatic steatohepatitis. Cytokines are classified as T helper 1 (Th1) and T helper 2 (Th2) subtypes. The main proinflammatory Th1 cytokines are tumor necrosis factor-alpha (TNF-a), interleukin (IL)-1, IL-6 and interferon; whereas the main Th2 anti-inflammatory cytokines are IL-4 and IL-10. In general, Th1 cytokines induce Th1 cytokines and inhibit Th2 cytokine production and vice versa. Normally, there is a balance between proinflammatory and anti-inflammatory cytokines. IL-6 though initially considered as a hepatoprotector in liver steatosis, capable of reducing oxidative stress and preventing mitochondrial dysfunction, was however was found to be a key element in the acute phase response, mediating the synthesis of several acute phase proteins. It has been seen that not only the cytokine itself but also its soluble receptor is significantly increased in patients with NASH. TNF-α receptor polymorphism is one
The present study is to investigate the relationship of Vitamin D status and Immunomodulators (TNF-α and IL-1α, IL-4, IL-6, IL-10, IL-17α) as the indicators of NAFLD in middle aged tribal subjects of Tripura. The objectives were to determine the status of 25(OH)D concentration in middle aged non-alcoholic tribal subjects of Tripura with and without fatty liver disease and to evaluate whether the risk of development of NAFLD in these subjects has any association with the level of Immunomodulators and Vitamin D status.

Materials and Methods

The study is an analytical cross-sectional study where a consecutive type of sampling was employed. In this study, a total of 94 tribal subjects from different communities across the state fulfilling inclusion and exclusion criteria and having ultrasound evidence of fatty liver were taken as cases. An equal number of age, sex and demography matched subjects from same tribal communities but without evidence of fatty liver on ultrasonography were taken as the control population. Cut of BMI: > 23 and 25 kg/m² for overweight and obesity respectively as per Asia Pacific Guidelines were implemented.

Sample size for this study has been calculated by the following formula:

Where:
1. \( n \) = sample size
2. \( r \) = ratio of cases and controls (in our study number of cases and controls being equal, \( r = 1 \))
3. \( Z_{e} \) = 0.84 for power of the study at being 80%
4. \( Z_{\alpha/2} = 1.96 \) for level of significance at 0.05
5. \( S_{1} = 9.2 \) (Standard deviation of case)
6. \( S_{2} = 9.7 \) (Standard deviation of control)
7. \( d = \) Difference between means (20.5 – 14.8 = 5.7)\(^{a}\)

For power of the study at 80%, and level of significance taken at 0.05, \( Z_{e} = 0.84 \) and \( Z_{\alpha/2} = 1.96 \), the required sample size was found to be 90. Finally, 94 subjects with NAFLD were included in the study as cases with a similar number of age, sex and demography matched healthy volunteers as control subjects.

Inclusion Criteria

1. Referred for assessment of abnormal liver function test (LFT) or hepatic steatosis detected by ultrasonography.
2. Age between 20 to 40 years
3. Alcohol consumption less than (40 gm/week)
4. Willing to participate in the study

Exclusion Criteria

1. Final diagnosis other than NAFLD
2. Secondary causes of steatohepatitis and drug induced liver disease
3. Any case of chronic liver disease

Height, weight, waist and hip circumference and blood pressure of each subject were measured at the baseline visit. Enzyme linked Immunosorbent Assay (ELISAs) was used to measure the serum concentration of TNF-α and IL-1α, IL-4, IL-6, IL-10, IL-17α in the NAFLD and control subjects. Chemiluminescent assay was used to determine the serum levels of 25(OH)D. Liver ultrasonography (US) scanning was performed to assess the degree of steatosis. All US were performed by the same operator who was unaware of the aims of the study and blinded to laboratory values using a US apparatus equipped with a convex 3.5 MHz probe.

SPSS version 20 statistical package was used to perform the analysis. Students T-test for continuous variables and \( \chi^2 \) test for categorical variables were used to compare mean values between two independent groups. 25(OH) Vitamin-D, TNF-α and IL-1α, IL-4, IL-6, IL-10, IL-17α were analyzed as continuous variables. Data is shown as mean ± standard X deviation. Receiver operator characteristic (ROC) curves were carried out to ascertain the usefulness of each of 25(OH)D, IL-6 and TNF-α as predictors of NAFLD in the whole study. Binomial logistics regressions were carried out to ascertain whether each of 25(OH)D, TNF-α and IL-1α, IL-4, IL-6, IL-10, IL-17α could independently predict the occurrence of NAFLD in the obese and non-obese subgroups. For all the above, a P-value <0.05 was considered statistically significant.

The study was funded by ICMR and the study protocol was approved by the Human Ethics Committee of the Tripura Medical College and Dr. BRAM Teaching Hospital and written informed consent was obtained in all cases.

Results

Of the 188 subjects who had enrolled in the study, maximum number (55/188, 29.3%) belonged to the age group between 36 to 40 years. The mean age of the study population was 33.23 years with a standard deviation of 6.916 years. Of the 188 subjects, 67 (35.6%) were male where as 121 (64.4%) subjects were female. 62.23% (117/188) subjects were obese while 37.77% (71/188) were non-obese. Of the obese subjects 68.38% (80/117) had fatty liver on ultrasonography whereas, of the non-obese subjects, only 19.72% (14/71) had fatty liver. Presence of obesity was significantly associated with presence of fatty liver (odds ratio: 8.80, 95% CI: 4.36 – 17.77, p < 0.001). There was a significant difference (Table 1) between the mean BMI of the cases and the controls (25.70 ± 2.93 kg/m² vs 23.09 ± 3.17 kg/m²; p < 0.001). 55.85% (105/188) subjects had subnormal levels of 25(OH) Vitamin D, either insufficiency (86/188, 45.74%) or deficiency (19/188, 10.11%). 44.15% (83/188) subjects had normal levels of 25(OH) Vitamin D. Of the 105 subjects with subnormal levels of 25(OH) Vitamin D, 76 (72.38%) had liver fat on ultrasonography where as,
Table 1: Difference between mean values of parameters between the cases and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Case (Mean + SD)</th>
<th>Control (Mean + SD)</th>
<th>Level of Significance (p Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH) Vitamin D</td>
<td>17.21 ± 6.34 ng/ml</td>
<td>26.56 ± 10.63 ng/ml</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-1a</td>
<td>11.50 ± 2.75 Pg/ml</td>
<td>8.28 ± 2.08 Pg/ml</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.69 ± 0.43 Pg/ml</td>
<td>0.84 ± 0.36 Pg/ml</td>
<td>0.009</td>
</tr>
<tr>
<td>IL-6</td>
<td>2.99 ± 1.11 Pg/ml</td>
<td>2.22 ± 1.08 Pg/ml</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-10</td>
<td>6.50 ± 2.76 Pg/ml</td>
<td>5.23 ± 2.67 Pg/ml</td>
<td>0.002</td>
</tr>
<tr>
<td>IL-17</td>
<td>5.33 ± 2.22 Pg/ml</td>
<td>3.64 ± 1.99 Pg/ml</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNF-α</td>
<td>6.99 ± 2.81 Pg/ml</td>
<td>5.40 ± 3.08 Pg/ml</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI</td>
<td>25.71 ± 2.93 kg/m²</td>
<td>23.09 ± 3.17 kg/m²</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2: Results of receiver operator curves

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC</th>
<th>p Value</th>
<th>95% CI</th>
<th>Cut off value</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1a</td>
<td>0.833</td>
<td>&lt;0.0001</td>
<td>0.772 – 0.883</td>
<td>9.35 Pg/ml</td>
<td>74.47%</td>
<td>86.17%</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.703</td>
<td>&lt;0.0001</td>
<td>0.632 – 0.767</td>
<td>2.08 Pg/ml</td>
<td>78.72%</td>
<td>58.5%</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.634</td>
<td>&lt;0.0001</td>
<td>0.561 – 0.703</td>
<td>3.80 Pg/ml</td>
<td>81.91%</td>
<td>41.49%</td>
</tr>
<tr>
<td>IL-17</td>
<td>0.746</td>
<td>&lt;0.0001</td>
<td>0.678 – 0.807</td>
<td>3.60 Pg/ml</td>
<td>73.12%</td>
<td>75.53%</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.694</td>
<td>&lt;0.0001</td>
<td>0.623 – 0.739</td>
<td>5.50 Pg/ml</td>
<td>61.70%</td>
<td>74.19%</td>
</tr>
</tbody>
</table>

of the 83 subjects with normal levels of 25(OH) Vitamin D, only 18 (21.69%) had fatty liver. There was a significant relationship between level of 25(OH) Vitamin D and fatty liver. p < 0.001 (Odds ratio: 9.46, 95% CI: 4.82 – 18.59). Figure 1A shows the relationship.

The mean serum 25(OH) Vitamin D level in the cases that is those with fatty liver was 17.21 ng/ml ± 6.34 ng/ml while the mean serum 25(OH) Vitamin D level in the control group was 26.56 ng/ml ± 10.63 ng/ml (Table 1). There was a significant difference (p < 0.001) between the mean serum 25(OH) Vitamin D of the cases and the controls.

There was a significant difference (p < 0.001) between the mean serum TNF-α levels of the cases and the controls (6.99 ± 2.81 Pg/ml vs 5.40 ± 3.08 Pg/ml). (Figure 2C)

Analysis of ROC curve for 25(OH) D showed an AUROC curve in NAFLD group (AUC=0.790; 95% CI[0.724 – 0.846], p < 0.0001) (Figure 2D). The optimal cut-off value of 25(OH)D for NAFLD was 20.75 ng/ml below which NAFLD could be predicted with a sensitivity of 84.04% and a specificity of 68.09%. The results of the analysis of ROC curve for the rest of the parameters have been illustrated in Table 2.

Binomial logistic regressions showed that low serum 25(OH)D [OR: 0.87 (95% CI: 0.83 – 0.92), p = 0.0001]and high serum IL-1a [OR: 1.52 (95% CI: 1.26 – 1.84), p < 0.0001] were independently associated with the risk of NAFLD in the study population. Table 3 depicts the scenario.

**Discussion**

The study sample was selected on a strict 1:1 pattern. While most of the other similar studies have not followed a 1:1 sampling, studies conducted by Jablonski et al.10 and that conducted by Targher G et al.11 have taken equal number of matched cases and controls.

Majority of the subjects, (62.23%) subjects were obese and majority (68.38%) of the obese subjects had fatty liver on ultrasonography. Presence of obesity was significantly associated with presence of fatty liver. There was a significant difference between the mean BMI of the cases and the controls.

This study revealed a significant relationship between level of 25(OH) Vitamin D and fatty liver. The mean serum 25(OH)D levels in the cases was significantly lower than that found in the control subjects. The results were in unison with those seen in the studies conducted by Wang D et al.,12 Zhai HL et al.,13 Wang X et al.14 Kucukazman et al15 demonstrated that in contrast to the control group, the cases with NAFLD in their study had significantly lower levels of 25(OH)D (12.3 ± 8.9 ng/ml vs 20.0 ± 13.6 ng/ml, p<0.001). Hao Y et al.,16 demonstrated similar results in their study where vitamin D levels were significantly lower in the NAFLD group.
and Black et al. 20 also showed similar results. Studies conducted by Targher G et al. 11 made a mention of IL-6 to be increased significantly greater in the NAFLD group than in the non-NAFLD group. However, in order to come to a definite conclusion, further studies involving larger number of subjects and long term follow ups are needed.

Acknowledgements

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References


Conclusion:

Patients presenting for TB retreatment have distinct demographic and clinical characteristics, important differences in retreatment outcomes in relation to different parameters. So, new country-specific strategies are required to identify and address risk factors for retreatment cases and factors responsible for poor outcome of these cases.

Background:

To evaluate the risk factors for retreatment failure, default or relapse and factors responsible for treatment outcome in retreatment cases may help in planning country-specific prevention strategies.

Abstract

Content and Risk of Non-Alcoholic Fatty Liver Disease


