A Study of Endothelial Dysfunction in Patients of Non-Alcoholic Fatty Liver Disease

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Abstract

Introduction: The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing worldwide rapidly and is regarded as the hepatic manifestation of metabolic syndrome. The present study was undertaken to study the endothelial dysfunction by flow mediated vasodilation in NAFLD patients.

Material and Methods: 32 cases and 16 age and sex matched controls were included in the study. Flow mediated vasodilation of the brachial artery was studied in both cases and controls. Anthropometric, clinical and biochemical assessment was also done.

Results: It was found that NAFLD patients had a significant endothelial dysfunction as assessed by flow mediated vasodilation as compared with controls. Percentage change in FMD among NAFLD patients (13.54±3.65%) was found to be lower than that in controls (16.84±4.61%) and difference was found to be statistically significant (p 0.010).

Conclusion: From the present study it can be concluded that NAFLD patients have significant endothelial dysfunction even in the absence of traditional risk factors of cardiovascular disease.

Editorial Viewpoint

• Non-alcoholic fatty liver disease is regarded as the hepatic manifestation of metabolic syndrome.
• This study finds NFLD patients have significant endothelial dysfunction even in the absence of traditional risk factors of cardiovascular disease.

Procedure for Measurement of Flow Mediated Vasodilation (FMD)

A longitudinal section of the brachial artery was analysed; (Medial epicondyle was used as anatomical landmark for brachial artery). USG machine with high resolution (B) scan 7.5Hz linear accelerator was used to assess brachial artery diameter and its changes.

Flow mediated vasodilation (FMD), which reflects endothelium dependent vasodilation, was calculated as the percentage increase in diameter from baseline to the maximum value which is obtained after the cuff deflation using the following formula:

\[
FMD = \frac{d_2-d_1}{d_1} \times 100
\]

where \(d_2\) = Brachial artery diameter at 5 min post deflation and \(d_1\) = Base line brachial artery diameter

Introduction

Non alcoholic fatty liver disease (NAFLD) is fast attaining the status of being the most common disease throughout the world. The prevalence is as high as 20-30% of general population in western countries¹⁴ while in India the prevalence in various studies varies from 9-32% in different studies.⁵-⁷ NAFLD is regarded by many to be the hepatic manifestation of metabolic syndrome and therefore it may be linked to cardiovascular disease. Since there are few studies on endothelial dysfunction in NAFLD we decided to undertake this study to assess endothelial function in NAFLD patients by flow mediated vasodilation.

Material and Methods

This was a case control study conducted in the department of medicine. All patients of NAFLD above 18 years of age who were diagnosed ultrasonographically were included in the study. Patients of NAFLD who had hepatitis B & C, autoimmune hepatitis, primary biliary cirrhosis, Wilson’s disease, chronic alcohol intake, diabetes, hypertension, dyslipidemia, on statin therapy and smokers were excluded from the study. Age and sex matched healthy individuals served as controls. A written informed consent was taken from all patients. The patients underwent a complete clinical examination, anthropometric measurements, laboratory tests and ultrasonography.

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Results

The present study was conducted in the Department of Medicine of a teaching hospital to study the endothelial dysfunction by flow mediated vasodilatation in patients of Non-alcoholic Fatty Liver disease. All patients more than 18 years of age, admitted to indoor/attending the OPD in the Department of Medicine, and ultrasonographically diagnosed as non-alcoholic fatty liver disease were enrolled in the study. Of these, 32 cases fulfilling the inclusion criteria and giving consent to be included in the study were included as Cases. 16 Age and Sex matched controls were also included in the study.

Age of patients included in the study ranged from 22 to 72 years. The mean age of Cases was 45.06±10.91 years and that of Controls was 45.06±12.71 years (Table 1).

The BMI of subjects included as Cases and Controls ranged from 20-24.50 kg/m^2 while difference in mean BMI of Cases (22.27±1.48 kg/m^2) and Controls (22.53±1.56 kg/m^2) was not found to be statistically significant.

Chief complaint of majority of cases of NAFLD was abdominal pain (53.13%) followed by fullness of abdomen (37.50%) and decreased appetite (9.38%).

No statistically significant differences in above hematological and biochemical variables of Cases and Controls was found (p>0.05) (Table 2).

Though brachial artery diameter at baseline of Controls (3.81± 0.16 mm) was found to be higher than that of Cases (3.78±0.17 mm) but difference in brachial artery diameter of Cases and Controls was not found to be statistically significant (p=0.533) (Table 3).

Range of Post-cuff deflation brachial artery diameter of Cases was 3.92-4.76 mm while that of Controls was 4.22-4.68 mm. Mean post-cuff deflation brachial artery diameter of Cases was 4.29±0.22 mm while that of Controls was 4.45±0.15 mm. Difference in post-cuff deflation brachial artery diameter of Cases and Controls was found to be statistically significant (p=0.013) (Table 3).

Range of change in Brachial artery in Cases was 0.2-0.90 mm while in Controls it was 0.36-0.93 mm. Mean brachial diameter change in Cases (0.51±0.14 mm) was found to be statistically significant. It was lesser than that in Controls (0.64±0.16 mm) (Table 3).

% Change in FMD among Cases (13.54±3.65%) was found to be lower than that in Controls (16.84±4.61%) and difference was found to be statistically significant (Table 4).

At baseline, statistically no significant difference was observed between two groups with respect to brachial artery diameter, however, post-cuff deflation diameter was found to be significantly lower in cases as compared to controls. On evaluating the change in brachial artery diameter too, it was found to be lower in cases as compared to controls for both absolute as well as percentage change (FMD%) (Table 5).

Discussion

Non-alcoholic fatty liver...
Although association between NAFLD and endothelial dysfunction as observed by flow mediated dilatation has been studied in various studies\(^{10-12}\) and some of these studies showed that irrespective of presence of components of metabolic syndrome (diabetes, obesity, dyslipidemia, hypertension), NAFLD itself was a strong predictor of endothelial dysfunction,\(^{10,12}\) thus opening a new dimension for exploration.

Endothelial dysfunction is a well established response to cardiovascular risk factors and precedes the development of atherosclerosis. Endothelial dysfunction is involved in lesion formation by the promotion of both the early and late mechanisms of atherosclerosis, including up-regulation of adhesion molecules, increased chemokine secretion and leukocyte adherence, increased cell permeability, enhanced low-density lipoprotein oxidation, platelet activation, cytokine elaboration, and vascular smooth muscle cell proliferation and migration. Endothelial dysfunction is a term that covers diminished production/availability of nitric oxide and/or an imbalance in the relative contribution of endothelium-derived relaxing and contracting factors. Also, when cardiovascular risk factors are treated the endothelial dysfunction is reversed and it is an independent predictor of cardiac events.

### Table 4: Comparison of % change in FMD among study population

<table>
<thead>
<tr>
<th></th>
<th>Mean ± S.D.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>13.54 ± 3.65</td>
<td>5.05 - 23.81</td>
</tr>
<tr>
<td>Controls</td>
<td>16.84 ± 4.61</td>
<td>9.09 - 24.80</td>
</tr>
<tr>
<td>Total</td>
<td>14.64 ± 4.25</td>
<td>5.05 - 24.80</td>
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\(t=2.699; p=0.010\)

### Table 5: Intergroup (cases-controls) change in brachial artery diameter from baseline and post- cuff deflation

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=32) Mean ± S.D.</th>
<th>Controls (n=16) Mean ± S.D.</th>
<th>Significance of difference 't' 'p'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline diameter</td>
<td>3.78 ± 0.17</td>
<td>3.81 ± 0.16</td>
<td>0.587 'p=0.560</td>
</tr>
<tr>
<td>Post-cuff diameter</td>
<td>4.29 ± 0.22</td>
<td>4.45 ± 0.15</td>
<td>2.614 'p=0.012</td>
</tr>
<tr>
<td>Change diameter</td>
<td>0.51 ± 0.14</td>
<td>0.64 ± 0.16</td>
<td>2.892 'p=0.006</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>13.54 ± 3.65</td>
<td>16.84 ± 4.61</td>
<td>2.702 'p=0.010</td>
</tr>
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\(t=20.910; p<0.001 \quad t'=15.994; p<0.001\)

In present study, majority of patients were above 40 years of age (59.4%). Mean age of patients was 43.06±10.91 years. In a study by Amarapurkar et al. (2007)\(^6\) it was reported that the prevalence of NAFLD was associated with age >40 years. However, they also found NAFLD prevalence to be associated with central obesity, elevated fasting blood sugar and raised liver functions. In the present study despite the absence of these risk factors, the age profile of patients did not show a change. However, in another study by Kim et al. (2004)\(^{13}\) mean age of patients with NAFLD was reported to be 53.2±9.8 years. However, the difference between two groups could be attributed to the difference in inclusion criteria. In present study, we included all the subjects above 18 years of age, however the inclusion criteria used by Kim et al. (2004)\(^{13}\) set the inclusion age to be 30 years. In the study of Mohammadi et al. (2011),\(^{10}\) who conducted the study in a design and patient selection criteria similar to ours, the mean age of patients was reported to be 38.7±14.952 years. In another study conducted by Colak et al. (2013)\(^{12}\) who also conducted their study in non-obese NAFLD patients reported the mean age to be 42.8±9.8 years. All these findings indicate towards a high age-related association of NAFLD, irrespective of the obesity status. With aging, the liver undergoes substantial changes in structure and function that are associated with significant impairment of many hepatic, metabolic and detoxification activities.\(^{14}\) Although NAFLD is not uncommon in children and teenagers yet in that situation it is mostly associated with obesity.\(^{15}\)

In present study, majority of patients were males (62.5%). Male to female ratio was 1.67:1. This finding is also in agreement with the observation made by Amarapurkar et al (2007)\(^6\) who found NAFLD to be more prevalent in males than females. They reported a male to female ratio of 1.81:1.

In the present study, among NAFLD patients, pain abdomen (53.13%) was the most common complaint followed by fullness of abdomen (37.50%) and decreased appetite (9.38%) respectively. To the best of our knowledge none of these complaints have a known relationship with cardiovascular risk and endothelial dysfunction.

In the present study, baseline brachial artery diameter was 3.78±0.17 mm in NAFLD cases.
and 3.81±0.16 mm in controls, thus showing no significant difference between two groups. However, post dilatation evaluation showed the mean values to be 3.92±4.76 mm in cases as compared to 4.22±4.68 mm in controls. Thus, flow mediated dilatation in NAFLD patients was 13.54±3.65% in NAFLD cases as compared to 16.84±4.61% in controls. Statistically these differences were significant too.

Reduction in endothelial function in NAFLD patients as compared to healthy controls has been observed in almost all the reviewed studies that have evaluated this relationship.

Recently, a lot of data from the Western literature has suggested the increased atherosclerosis and cardiovascular risk in patients with NAFLD but it is still a matter of debate whether NAFLD per se predisposes to these abnormalities or this is all happening because of the presence of metabolic abnormalities. Mohammadi et al (2011) remarked that in understanding the true pathophysiologic basis of NAFLD, the chicken and egg phenomenon persists, in that some authors believe that NAFLD can produce insulin resistance while others claim that insulin resistance is the major determinant of development and progression of fatty liver to nonalcoholic steatohepatitis.

Endothelium is conceived to be the largest endocrine gland in the body that secretes many transmitters in order to maintain homeostasis in the circulatory system. FMD is a noninvasive ultrasonographic method which is currently recognized as a useful technique for evaluating endothelial function. The basic mechanism for FMD is to observe vasodilatation by sonography after provoking ischemia by inflating blood pressure cuff. After brachial artery occlusion, endothelial nitric oxide is released and vascular smooth muscle relaxation occurs.

One of the early processes in the pathophysiology of atherosclerosis is impaired endothelial function. Impaired endothelial function quantified by FMD is a marker of increased cardiovascular risk, due to its correlation with impaired endothelial function in the coronary arteries.

The endothelium maintains normal vascular tone and blood fluidity, with no or little expression of proinflammatory factors under normal homeostatic conditions. Generally accepted cardiovascular risk factors like smoking, aging, hypercholesterolemia, hypertension, hyperglycemia, and a family history of premature atherosclerotic disease are all associated with alteration in endothelial function. This results in a chronic inflammatory process accompanied by a loss of antithrombotic factors and an increase in vasoconstrictor and prothrombotic products, in addition to abnormal vasoreactivity, therefore elevating risk of cardiovascular events. Studies have shown that NAFLD is also associated with chronic portal inflammation however this phenomenon is also shown to be increased with presence of different factors of metabolic syndrome, thus showing a probable inseparable relationship.

Federico et al (2016) have invoked the role of proinflammatory cytokines and low grade inflammation in NAFLD in causing endothelial dysfunction. Cytokine production and inflammation lead to inefficiency of mechanisms that underlie functional endothelial homeostasis.

However, there is ample evidence that metabolic syndrome factors like diabetes, hypertension, dyslipidemia, obesity vis-à-vis NAFLD can both coexist as well as remain independent of each other and despite having a common risk factor profile may or may not manifest, however, all these disorders affect the metabolic activity and independently have higher odds for cardiovascular risk.

The findings of present study confirmed that NAFLD as an independent marker has an atherogenic effect that affects the FMD values. One of the limitations of present study was a small sample size, owing to which the association between severity of NAFLD and FMD could not be assessed. Moreover a correlation between different grades of NAFLD and preclinical risk for metabolic syndrome factors could not be studied. It would be of interest to carry out a larger study in a sample having both NAFLD with Metabolic Syndrome factors and without Metabolic Syndrome factors as well as controls, in order to evaluate the cumulative effect of NAFLD on FMD.

Conclusions

The findings of the present study show that there is significant endothelial dysfunction, independent of other cardiovascular risk factors, in patients of nonalcoholic fatty liver disease in comparison to controls.

References

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