Role of Activin A as A Novel Marker for Diagnosis and Evaluation of Nonalcoholic Fatty Liver Disease

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ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD) is a disease gaining increasing interest worldwide. It ranges from simple nonalcoholic steatosis to nonalcoholic steatohepatitis (NASH), which is characterized by steatosis, inflammation and fibrosis, and may lead to liver cirrhosis and hepatocellular carcinoma.

Objectives: To study the role of activin A, a member of the transforming growth factor (TGF) superfamily, in diagnosis and evaluation of non-alcoholic fatty liver disease (NAFLD).

Patients and Methods: This study is a comparative case-control study. This study was carried out at Outpatient Clinics of Internal Medicine Department of Ain Shams University. The study included 90 patients that were divided into 3 groups. Group 1 that included 35 patients with NAS (steatosis) with exclusion criteria of intake of hepatotoxic drugs, group 2 that included 35 patients with fatty liver and elevated liver enzymes (NASH) and group 3 that included 20 patients as a control group.

Results: There was a high statistical significant difference between the studied groups as regards ALT, AST, total and direct bilirubin, platelets count, activin A, and Albumin. There was a high statistical significant difference between the studied groups as regards APRI score and FIB-4. There was high correlation between activin A and BMI, APRI score, FIB-4 and liver size with high significance (p < 0.001). Using activin A, it was shown that above 858.5, it can discriminate between NAFLD and non-NAFLD with level of sensitivity 100% and specificity 100%.

Conclusion: Serum activin A showed a trend towards progressive level increase from controls, to steatosis and NASH patients.

Keywords: Activin A, Non-alcoholic fatty liver disease.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is becoming one of the most frequent causes of impaired liver function, with an estimated incidence of over 20% in the Western world (1). NAFLD is a spectrum of disease that ranges from simple steatosis (fat accumulation) of the liver to non-alcoholic steatohepatitis (NASH) with inflammation and fibrosis, followed by extensive fibrosis and NASH-associated cirrhosis in the most advanced forms of NASH. While NASH implies a risk of progressive liver disease (2), simple steatosis may be regarded as a risk factor (3). NAFLD is characterised by triglyceride buildup in hepatocytes, necrosis, and apoptosis of these cells, as well as inflammatory and fibrogenic reactions in the liver, which can progress to cirrhosis.

In NASH, the two-hit model highlights the key early metabolic processes that contribute to fat buildup and, eventually, hepatic necrosis and inflammation (4). According to the two-hit model, identifying variables that cause hepatic fat accumulation as well as mediators that facilitate the hepatic shift from basic steatosis to NASH is critical. NAFLD is commonly linked to the metabolic syndrome, which includes obesity, dyslipidemia, and insulin resistance (IR) (5). Though these factors, as well as inflammation and oxidative stress (6), may predispose to NAFLD development, the mechanisms that underlie hepatic fat accumulation and triggering of hepatocyte injury and hepatic fibrosis in NASH are still largely unknown. Little is known, in particular, regarding the mediators that may cause an extensive hepatic fibrogenic response in certain people with NAFLD, leading to advanced NASH.

Activin A is a member of the transforming growth factor (TGF)-b superfamily (7) and was first identified as a follicle-stimulating hormone inducer (8). Studies have also suggested that activin A may have a role in the development of a variety of liver diseases, including acute liver damage, chronic viral hepatitis, and some hepatic cancers (9). We established that activin A has a role in NAFLD (10). The aim of the present study was to study the role of activin A, a member of the transforming growth factor (TGF) superfamily, in diagnosis and evaluation of non-alcoholic fatty liver disease (NAFLD).

PATIENTS AND METHODS

Study design: This study is a comparative case control study.

Setting: This study was carried out at Outpatient Clinics of Internal Medicine Department of Ain Shams University.


Target population: Group 1 included 35 patients with NAS (steatosis) with exclusion criteria of intake of...
hepatotoxic drugs, group 2 included 35 patients with fatty liver and elevated liver enzymes (NASH) and group 3 that including 20 patients as a control group.

**Inclusion criteria:** Middle aged patients (20-60 years), bright liver on ultrasound imaging and abnormal liver function tests for at least 6 months.

**Exclusion criteria:** Alcoholic patients, patients with autoimmune hepatitis, patients with viral hepatitis, diabetic patients, patients with liver failure, patients with hepatocellular carcinoma, hepatotoxic drugs.

**Sampling technique:** This study was performed on systematic random sampling.

**Methods:**

**Clinical assessment:** History; complete history taking including age, sex, residency, occupation, smoking or ex-smoker, presenting complaint, jaundice, itching, abdominal pain and presence of comorbidities. History of previous gastrointestinal bleeding.

**Clinical examination:** General examination and abdominal examination

**Body mass index measuring and Laboratory assessment (routine and general evaluation tests):**
Complete blood count (CBC): Hemoglobin (HB) % (g/dl), white blood cells (wbcs) (c/mm), platelet count (cmmm).

**Kidney function tests:** Blood urea (mg/dl) and serum creatinine (mg/dl).

**Liver profile:** Alanine amino transferase (ALT) (Iu/l), aspartate amino transferase (AST) (Iu/l), serum albumin (g/dl), total and direct serum bilirubin (mg/dl), prothrombin time (P.T) and I.N.R.

**Imaging:** All patients were submitted to abdominal ultrasonographic examination for the liver (size, surface, echogenicity, focal lesions, and biliary tree), Portal and splenic vein (diameters and patency), Splenic size, ascites, and abdominal masses.

**Human activin A Elisa:** we used BioVendor Human Activin A ELISA. standards, quality controls and samples are incubated at 37°C in microplate wells precoated with polyclonal anti-human activin A antibody. The kit measures human activin in serum and plasma (EDTA, citrate, heparin, USA).

**Ethical consent:**
An approval of the study was obtained from Ain Shams University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of the study. This work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

**Statistical analysis**
Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean ± standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at 5% level. Chi-square test was used for categorical variables, to compare between different groups.

**F-test (ANOVA)** for normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) (LSD) for pairwise comparisons. Kruskal Wallis test was used for abnormally distributed quantitative variables, to compare between more than two studied groups and Post Hoc (Dunn's multiple comparisons test) for pairwise comparisons. Spearman coefficient was used to correlate between two distributed abnormally quantitative variables. Pearson coefficient was used to correlate between two normally distributed quantitative variables. A P value ≤ 0.05 was considered significant.

**RESULTS**
Table (1) showed that in the steatosis group, there were 10 (28.6%) males and 72 (72%) females, with mean age of 35.89 ± 9.10 years with range 20-55 years. In the NASH group, there were 18 (51.4%) males and 17 (48.6%) females, with mean age of 35.17 ± 10.10 with range 21-54 years. In the control group, there were 8 (40%) males and 12 (60%) females, with mean age of 37.15 ± 10.18 with range 24-55 years. There was no statistically significant difference between the three studied groups as regards demographic data.

| Table (1): Comparison between the three studied groups as regards demographic data |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Age (years)** | **Steatosis group** | **NASH group** | **Control group** | **Test of sig.** | **P** |
| Min. – Max. | (n=35) | (n=35) | (n=20) | F=0.263 | 0.770 |
| Mean ± SD. | 20.0 – 55.0 | 21.0 – 54.0 | 24.0 – 55.0 | 35.89 ± 9.10 | 35.17 ± 10.10 | 37.15 ± 10.18 |
| **Sex** | | | | | |
| No. | % | No. | % | No. | % |
| Male | 10 | 28.6 | 18 | 51.4 | 8 | 40.0 | χ²=3.810 | 0.149 |
| Female | 25 | 71.4 | 17 | 48.6 | 12 | 60.0 |

χ²: Chi square test
F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test, (Tukey)
*p value comparing between the three groups
*: Statistically significant at p ≤ 0.05
There was high statistically significant difference between the studied groups as regards weight and BMI (Table 2).
Table (2): Comparison between the three studied groups as regard anthropometrics

<table>
<thead>
<tr>
<th></th>
<th>Steatosis group (n=35) Mean ± SD</th>
<th>NASH group (n=35) Mean ± SD</th>
<th>Control group (n=20) Mean ± SD</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>76.04 ± 9.70</td>
<td>92.14 ± 13.21</td>
<td>69.93 ± 6.21</td>
<td>33.759</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>p</td>
<td>p1&lt;0.001*, p2=0.107</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.83 ± 5.56</td>
<td>168.54 ± 6.0</td>
<td>168.65 ± 4.78</td>
<td>1.058</td>
<td>0.352</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.25 ± 2.44</td>
<td>32.38 ± 3.84</td>
<td>24.57 ± 1.70</td>
<td>51.143</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Finding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>p1&lt;0.001*, p2=0.005*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

χ²: Chi square test  
F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test, (Tukey)  
p: p value comparing between the three groups  
p1: p value comparing between Steatosis group and NASH group  
p2: p value comparing between Steatosis group and Control group  
*: Statistically significant at p ≤ 0.05

There was high statistically significant difference between the studied groups as regards liver size (Table 3).

Table (3): Comparison between the three studied groups as regards ultrasound

<table>
<thead>
<tr>
<th></th>
<th>Steatosis group (n=35) Mean ± SD</th>
<th>NASH group (n=35) Mean ± SD</th>
<th>Control group (n=20) Mean ± SD</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver size</td>
<td>13.97 ± 1.29</td>
<td>13.71 ± 1.36</td>
<td>9.85 ± 1.50</td>
<td>66.63</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Finding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>p1=0.712, p2&lt;0.001*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enlarged-echogenic</td>
<td>35 100.0</td>
<td>18 100.0</td>
<td>0 0.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Normal</td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>20 100.0</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

χ²: Chi square test  
F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test, (Tukey)  
p: p value comparing between the three groups  
p1: p value comparing between Steatosis group and NASH group  
p2: p value comparing between Steatosis group and Control group  
*: Statistically significant at p ≤ 0.05

There was statistically significant difference between the studied groups as regards ALT, AST, total and direct bilirubin, plts count, activin A and albumin (Figs. 1, 2 and 3).
There was high statistically significant difference between the studied groups as regards APRI score and FIB-4 (Figs. 4 and 5).

**Figure (2):** Comparison between the three studied groups as regards AST.

**Figure (3):** Comparison between the three studied groups as regard activin A.

**Figure (4):** Comparison between the three studied groups as regards APRI score.
Table (4): Correlation between Activin A and Some parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Activin A</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>$r_p=0.660$</td>
<td>$&lt;0.001^*$</td>
<td></td>
</tr>
<tr>
<td>APRI score</td>
<td>$r_s=0.695$</td>
<td>$&lt;0.001^*$</td>
<td></td>
</tr>
<tr>
<td>FIB-4</td>
<td>$r_s=0.678$</td>
<td>$&lt;0.001^*$</td>
<td></td>
</tr>
<tr>
<td>Liver size</td>
<td>$r_p=0.586$</td>
<td>$&lt;0.001^*$</td>
<td></td>
</tr>
</tbody>
</table>

$r_s$: Spearman coefficient, $r_p$: Pearson coefficient, $^*$: Statistically significant at $p \leq 0.05$

Using activin A, it was shown that above 858.5, it can discriminate between NAFLD and non-NAFLD with level of sensitivity 100% and specificity 100% (Table 5 & figure 6).

Table (5): Roc curve analysis for the use of activin A for the discrimination between NAFLD and non-NAFLD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cut-off</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMA conc.</td>
<td>$&gt;858.5$</td>
<td>1.000</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Fig (6): Roc curve analysis for the use of Activin A for the discrimination between NAFLD and non-NAFLD.
DISCUSSION

In this study, we found that there was no statistically significant difference between the three studied groups as regards age (years) and sex. In the steatosis group, there were 10 (28.6%) males and 72 (72%) females, with mean age of 35.89 ± 9.10 with range of 20-55 years. In the NASH group, there were 18 (51.4%) males and 17 (48.6%) females, with mean age of 35.17 ± 10.10 with range of 21-54 years. In the control group, there were 8 (40%) males and 12 (60%) females, with mean age of 37.15 ± 10.18 with range of 24-55 years. Yndestad et al. (10) showed that in the steatosis group, there were 15 (46.8%) males and 17 (53.2%) females, with mean age of 44.7 ± 12.5. In the NASH group, there were 25 (65.7%) males and 13 (34.3%) females with a mean age of 43.7 ± 12.5. In the control group, there were 16 (53.3%) males and 14 (46.7%) females, with mean age of 41.8 ± 9.1.

Polyzos et al. (11) showed that thirty-one patients with biopsy proven NAFLD (15 with SS [aged 53.9 ± 2.6; 10 women] and 16 with borderline or definite NASH [aged 53.9 ± 2.9; 13 women]), 24 lean (aged 54.2 ± 1.6; 20 women) and 28 obese controls (aged 52.6 ± 1.6; 20 women) were included in this study.

In our study, there was high statistically significant difference between the studied groups as regards weight and BMI and insignificant difference regarding height. In agreement with our results, Yndestad et al. (10) reported that after adjustment for sex, BMI, and age, the diagnosis of NAFLD was still a significant predictor of activin A (P = 0.002). Similar results were reported by Polyzos et al. (11) who reported that BMI and waist circumference were statistically lower in lean controls, but similar among patients with SS, NASH and obese controls.

In the present study, that there was high statistically significant difference between the studied groups as regards ALT, AST, bilirubin, platelets count, activin A and significant difference between the studied groups as regards albumin. Matching our results, Polyzos et al. (11) showed that there were statistically significant differences in ALT, AST, ALT to AST ratio, HDL-C, LDL-C, triglycerides, uric acid, insulin, HOMA-IR, leptin and adiponectin between groups, with NASH generally being the group with the higher metabolic burden.

In our study, we found that there was high statistically significant difference between the studied groups as regards APRI score, FIB-4. Peleg et al. (12) showed that the APRI score was a good predictor for advanced fibrosis in NAFLD patients (area under the ROC curve 0.8307). Although, it was modestly inferior as compared to the well validated FIB-4 score (area under the ROC curve 0.8959). The predictive value of APRI score in NAFLD patients was inferior as compared to its predictive value in HCV patients (area under the ROC curve of 0.8307 versus 0.9965). In contrast to FIB-4, APRI score was not a good discriminator between intermediate stages of fibrosis in NAFLD patients. Tapper et al. (13) illustrated that APRI >1 was the most significant predictor of advanced fibrosis (OR 3.85; 95% CI: 1.55–9.59). In patients without ultrasound-detected steatosis, 20% had advanced fibrosis and 16.7% had active NASH.

In this study, we found that there was high correlation between activin A and BMI, APRI score, FIB-4 and liver size with high significance (P < 0.001). In agreement with our results, Polyzos et al. (11) showed that within group of NAFLD patients (n = 31), activin A was positively correlated with BMI, AST to ALT ratio, glucose, insulin, HOMA-IR, and leptin.

Similar results were reported by Yndestad et al. (10) who showed that activin A levels were related to age, sex, BMI (in women), and to the occurrence of the metabolic syndrome in patients with NAFLD. The presence of NASH (as opposed to simple steatosis) was still a significant predictor of activin A, even after adjustment for these parameters (P = 0.03).

In our results, we found that using activin A; it was shown that above 858.5, it can discriminate between NAFLD and non-NAFLD with level of sensitivity 100% and specificity 100%. Yndestad et al. (10) showed that NAFLD patients are characterized by increased serum levels of activin A, with particularly high levels in those with NASH, accompanied by an increased activin A – follistatin mRNA ratio within the liver of NAFLD patients, potentially reflecting higher hepatic activin A bioactivity. Our findings suggest that NAFLD should be added to the list of hepatic disorders, which could involve activin A-mediated mechanisms. Previously, Patella et al. (14) showed that activin A had been reported to inhibit hepatocyte growth by downregulating hepatocyte replication and inducing apoptosis, as well as by inducing the production of extracellular matrix during liver fibrosis. Jones et al. (15) showed that although serum levels of both activin A and follistatin were increased in NAFLD patients compared to that in controls, there was a relatively higher increase in follistatin, resulting in a lower serum activin A–follistatin ratio in NAFLD. This finding may apparently seem in conflict with our finding of the increased activin A–follistatin ratio within the liver in NAFLD. However, as activin A is primarily a paracrine / autocrine-acting cytokine, it might be more relevant biologically to analyze the activin A – follistatin ratio within a particular tissue, such as the liver, rather than to calculate the ratio in peripheral circulation reflecting altered production of these mediators in several compartments throughout the body (e.g., adipose tissue and vascular bed). Yet, although the cellular sources of serum levels of activin A and follistatin in NAFLD remains to be defined fully, our study suggests that serum levels of activin A, as opposed to the activin A–follistatin ratio and follistatin, may be a non-invasive marker of NASH- and NASH-related liver fibrosis.
Rodgarkia-Dara et al. (9) showed that the pathogenesis of NAFLD is incompletely understood, but hepatocyte injury, inflammation, and fibrosis seem to be important pathogenic features of NAFLD-related disorders, and activin A could potentially modulate all these interacting processes. Thus, activin A has been reported to negatively regulate the hepatic cell number by inhibiting cell replication and inducing apoptosis effects with potential relevance to the development and progression of NAFLD. Activin A has also been linked to hepatic inflammation, at least partly because of its ability to induce macrophage activation. Russell et al. (16) showed that the role of activin A in hepatic disorders may be far more complex. Hence, both pro- and anti-inflammatory effects that have been described for activin A is rapidly induced during systemic inflammation, and can antagonize interleukin-6-mediated effects within hepatocytes.

Parsons et al. (17) showed that activin A has previously been reported to induce hepatic production of extracellular matrix, at least partly involving activin A-mediated induction of fibronectin and collagen synthesis in hepatic stellate cells. In this study, we extended these findings in several ways. First, we showed that activin A may promote collagen synthesis also in hepatocytes. Smith et al. (18) showed that in addition to activin A, TGF-1 is a potent inducer of fibrogenesis in hepatic stellate cells, and our finding of a marked activin A-mediated increase of TGF-1 in hepatocytes in vitro further links activin A to the development of hepatic fibrosis. Finally, we also showed that activin A is a potent inducer of MMP activity in hepatocytes. Whereas, enhanced MMP activity could contribute to the resolution of fibrosis, MMP activity could also be a stimulus for fibrotic activity in hepatic stellate cells. De Bleser et al. (19) showed that although the role of activin A in NAFLD and other fibrotic hepatic disorders seems complex, it is tempting to hypothesize that it primarily promotes hepatic fibrosis and remodeling. Nonetheless, activin A seems to be linked to the fibrotic process, and our findings of a significant relationship between serum levels of activin A and the degree of hepatic fibrosis in NASH, suggest that activin A could be evaluated as a non-invasive marker of liver fibrosis in NAFLD. This study had some limitations, such as the lack of longitudinal data. In addition, we have no information on the occurrence of the metabolic syndrome in the control group, and these individuals should ideally have been examined with ultrasound to rule out low-grade steatosis. Nevertheless, our findings of increased serum levels of activin A in NAFLD, with particularly high levels in NASH and overt fibrosis, suggest that serum levels of activin A could be a marker of NAFLD-related disorders. It is possible that the strong relationship between activin A and NAFLD/NASH could reflect the ability of serum levels of activin A to mirror several processes with relevance to NAFLD, such as systemic inflammation, disturbed lipid and glucose metabolism, and fibrogenesis. The markedly increased hepatic activin A–follistatin ratio in these patients may suggest increased hepatic activin A bioactivity in NAFLD, supporting a role for activin A in NAFLD not only as a marker but also as a mediator. Although, activin A may have potentially important effects in NAFLD, involving effects on fibrosis and lipid accumulation, our findings also illustrate the pleiotropic and complex nature of activin A-mediated effects in the liver.

CONCLUSION

Serum activin A showed a trend towards progressive level increase from controls, to steatosis and NASH patients. Prospective studies are needed to confirm the hypothesis rose herein, before mechanistic studies attempt to elucidate mechanism and prove causality.

Financial support and sponsorship: Nil.
Conflict of Interest: Nil.

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