Study of Adiponectin Promotor Methylation status in patients with Non Alcoholic Fatty Liver Disease

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Abstract
Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of liver disease globally, and it is likely to overtake alcoholic liver disease as the primary cause of end-stage liver disease in the coming decades. It affects both adults and children. In light of the significant hurdles in the prevention and control of NAFLD, recent research has shown that detecting adiponectin promotor methylation may be useful in NAFLD diagnosis, prognosis, and treatment. Adiponectin (an adipose tissue hormone) induces apoptosis in hepatocytes, which has an anti-fibrotic effect. Several samples from patients with NAFLD have been shown to exhibit adiponectin promotor methylation (NAFLD) so it can be used as a non-invasive tool for NAFLD diagnosis.

Keywords: Non Alcoholic Fatty Liver Disease, Adiponectin, Methylation.

1. Introduction
After all other liver etiologies have been ruled out, non-alcoholic fatty liver disease (NAFLD) is caused by fat accumulation in the liver [1]. Hepatic fat accounts for more than 5–10% of entire liver weight in NAFLD. The majority of patients with NAFLD have merely an increase in liver fat (simple steatosis). Nonalcoholic steatohepatitis (NASH) is a disorder in which some individuals develop hepatic inflammation, and up to 20% of patients acquire progressive hepatic fibrosis, which can lead to liver cirrhosis or failure, as well as hepatocellular cancer [2].

NAFLD is a silent disease that affects the Egyptian people. It affected 20%–30% of the general population worldwide, but it can affect up to 50% of the population in obese people or those with diabetes mellitus. According to previous studies, steatosis is found in 70% of obese patients and 35% of non-obese patients, while NASH is found in 18.5 percent of obese patients and 3% of non-obese patients. Fatty liver disease might affect up to 75 percent of patients with type 2 diabetes mellitus (DM2) [3].

The total incidence of NAFLD in adolescents has reached about 10%, with 17% of teenagers and 40%–70% of obese youngsters suffering from the disease [4].

NAFLD pathogenesis has been linked to a number of risk factors, including advanced age, obesity, insulin resistance, and hyperlipidemia, as well as the involvement of pro- and anti-inflammatory cytokines [5].

A number of recent results have highlighted the importance of adipose tissue as an active endocrine organ that produces adipokines such as adiponectin (APN), leptin, resistin, and visfatin, all of which are involved in the progression and pathogenesis of NAFLD [6].

Hepatocytes respond to adiponectin by increasing free fatty acid oxidation while decreasing gluconeogenesis, FFA inflow, and de novo lipogenesis. Hepatocytes are protected against apoptosis by it. It has anti-inflammatory and anti-fibrotic properties by acting on Hepatic stellate cells (HSC), Kupffer cells, and perhaps sinusoidal cells. It works to reduce inflammation by inhibiting pro-inflammatory cytokines (TNF-α and IL-6) and activating anti-inflammatory cytokines (IL-10) [7].

Adiponectin's anti-fibrotic activity is primarily done by inhibiting HSC activation and proliferation while inducing apoptosis. TGF-β, connective tissue growth factor, and collagen are all downregulated by adiponectin, which favours matrix breakdown [8].

A liver biopsy is the gold standard for diagnosing NASH. This treatment, on the other hand, is invasive, pricey, and linked to uncommon but potentially dangerous consequences and sampling errors; thus, it is ineffective as a screening tool [2]. As a result, detecting adiponectin promotor methylation could be useful in NAFLD diagnosis, prognosis, and treatment.

2. Subject and methods
This study was carried out between July 2018 and July 2021 after approval of the study scheme by the research ethical committee of Benha Faculty of Medicine and obtaining informed consent from the included subjects. The study included 49 subjects of both sex selected from Endemic Medicine Department, Faculty of Medicine, Cairo University Hospital.

The subjects were categorized into 2 groups: patient group: included 34 patients diagnosed as non-alcoholic fatty liver disease patients by clinical, radiological and histopathological examinations examinations and control group: included 15 persons, age and sex matched, with normal liver.

All patients were subjected to full history taking with attention to: special habits including, obesity and sedentary life and many investigations, diagnostic Liver biopsy for histopathology and Methylation specific polymerase chain reaction (MSP) for detection of adiponectin promotor methylation status.

Blood samples were obtained from all individuals. Blood samples were collected into EDTA vacutainers. From each blood sample (1 ml) was transferred immediately into Eppendorf tubes to be stored at -
80°C, for later processing. Extraction of DNA from
Peripheral blood samples: using QIAmp DNA blood
mini kit (QIAGEN, Germany) according to
manufacturer's instructions. Bisulfite treatment of
extracted DNA was done by using The EZ DNA
Methylation-Gold Kit (ZYMO RESEARCH, USA),
according to manufacturer's instructions. Methylation
specific PCR by using HotStarTaq Master Mix Kit
(QIAGEN, Germany) and Specific primer sets for
either methylated or non-methylated products of
adiponectin gene promoter. The PCR product was
separated by gel electrophoresis, stained with ethidium
bromide and visualized by UV irradiation, for detection
of specific bands.

2.1. Statistical analysis
The collected data were summarized in terms of
mean ± Standard Deviation (SD) and range for
quantitative data; and frequency and percentage for
qualitative data. Comparisons between cases and
control were carried out using the student T-test, to
compare quantitative data between two groups and
Chi- squared (χ²) test, to compare proportions of two or
more groups. Pearson correlation was used to estimate
the correlation between ADPN-M, ADPN-U and age of
the studied group. The corresponding test statistics
were calculated and the corresponding P-values were
obtained. P-value 0.05 was considered statistically
significant, while P-value > 0.05 was considered
statistically non-significant. Analysis is performed
using the Statistics Program for Social Sciences (SPSS)
and Microsoft Office Excel is used for the data
processing and data analysis.

3. Results
49 persons were included in the study. 34 patients
were diagnosed with NAFLD while the remaining 15, having no evidence of
NAFLD, were included as controls.

Demographic data of studied groups
The baseline characteristics of the study
population are presented in Table-1. There was
insignificant difference between cases and control
groups regarding age (p=0.937), there is significant
difference between cases and control groups regarding
gender (p=0.022).

Table (1) Demographic data of the studied groups.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>NAFLD Group (n = 34)</th>
<th>Control Group (n = 15)</th>
<th>Test value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean± SD</td>
<td>43.88± 9.51</td>
<td>43.60± 14.98</td>
<td>T = 0.080</td>
<td>0.937</td>
</tr>
<tr>
<td>Median</td>
<td>41.50</td>
<td>47.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>27.0 - 64.0</td>
<td>19.00 - 64.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>8</td>
<td>X² = 5.25</td>
<td>0.022</td>
</tr>
<tr>
<td>Female</td>
<td>27</td>
<td>27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Methylation status of ADPN-M of studied groups
ADPN-M, was studied and Methylation status was analyzed in blood samples. The Methylation status was
significantly higher (p< 0.003) in NAFLD patients as compared to controls table (2).

Table (2) Comparison between the studied groups regarding degree of methylation.

<table>
<thead>
<tr>
<th>METH % (ΔΔCt)</th>
<th>NAFLD Group (n = 34)</th>
<th>Control Group (n = 15)</th>
<th>Test value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean± SD</td>
<td>53.68± 18.82</td>
<td>40.03± 10.98</td>
<td>3.18</td>
<td>0.003</td>
</tr>
</tbody>
</table>

SD: Standard deviation, P 0.05 ≤ significant, P > 0.05 non-significant, analysis done by independent samples
Student T test.

4. Discussion
NAFLD is a global health issue that affects over
25% of the world's population. The Middle East has the
greatest prevalence rate of NAFLD (31.79%), while
Africa has the lowest incidence rate (13.48 percent) (9).

In Egypt, a cross-sectional study showed that the
prevalence of NAFLD in children and adolescents was
15.8%, and the prevalence of NAFLD increased with
age, from less than 20% under 20 to over 60 for more
than 40% of the year, NAFLD is more common in men
(31%) than in women (16%) [9].

Although liver biopsy is the gold standard for
diagnosing NAFLD, it is an intrusive operation with
possible risks and complications. As a result, non-
invasive diagnostic methods as alternatives to liver
biopsy have been proposed [10].

The early identification of high-risk patients by the
measurement of a number of specific biomarkers is
critical to a successful preventative programme. As
treatment options for NASH patients at risk of
progression are applied, biomarkers are becoming
increasingly important for screening and identifying

The subject of genetic variables in NAFLD
research is continuously expanding, NAFLD and its
severity have been linked in many studies to serum

adipokines, a group of bioactive proteins released by adipose tissue that have anti-inflammatory and insulin-resistance properties. Adiponectin has been shown to protect against NAFLD in several trials [10]. Adiponectin is an adipose tissue-derived hormone that regulates glucose and lipid metabolism to influence whole-body energy homeostasis. Adiponectin improves insulin sensitivity in metabolic tissues by boosting glucose consumption and fatty acid oxidation. Obesity and obesity-related metabolic disorders such as insulin resistance, T2D, and cardiovascular disease are inversely linked with adiponectin blood levels [12].

In our study, there is adiponectin promoter hypermethylation status in patient with NAFLD, this is in line with previous research which shown that DNA hypermethylation of the adiponectin promoter inhibits adiponectin expression and, as a result, decreases its activity and exacerbates metabolic disorders in obese people [13]. Hypoadiponectinemia was found in women with GDM, according to another investigation. In maternal fat and blood cells, significant changes in locus-specific DNA methylation were detected. The methylation of DNA in GDM offspring was changed [14]. It could be concluded that the hypermethylation status of adiponectin promoter in NAFLD patients compared to the controls suggests their role as diagnostic non-invasive markers. The results of this study revealed that ADPN-M in blood sample is a potentially useful blood biomarker for early diagnosis of NAFLD.

5. Conclusion
It could be concluded that hypermethylation status of adiponectin promoter in NAFLD patients compared to controls suggests their role as diagnostic non-invasive markers, the result of this study revealed that ADPN-M in blood sample is a potentially useful blood biomarker for early diagnosis of NAFLD.

References