Relation of retinol binding protein4, visfatin and vitamin a in obese and non obese Iraqi patients with non alcoholic fatty liver disease

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Abstract---One of the most common public liver diseases over the world is fatty liver which contain alcoholic and non-alcoholic fatty liver. One-fourth among general population are impact Non-Alcoholic Fatty Liver Disease (NAFLD) in the worldwide. Retinol binding protein 4 (RBP4) is known as an adipokine, mainly synthesized and secreted from the liver and form adipose tissues. RBP4 acts as a transporter and specifically bound to retinol from liver to others tissues. Visfatin is an adipocytokine and mainly produced from visceral fat tissue, skeletal muscles as well as liver. Vitamin A absorbed, transported as retinyl esters to the liver then hydrolyzed to the retinol form and storage in hepatic stellate cells (HSCs) after reesterified with riglycerides. Hepatic retinyl esters are hydrolyzed to retinol and binds to its carrier RBP4 then transport to the target tissues. Methods: Ninety serum blood samples were collected and distributed into three groups: First group (G1) contained of (34) patients of obese NAFLD. Second group (G2) contained of (26) non obese NAFLD and control patients (G3) contained of (30) patients. Ultrasound was used to confirm the diagnosis of nonalcoholic fatty liver disease by examining the abdomen. Adiponectin and visfatin kits were used Sandwich ELISA principle. Vitamin A was measured by competitive inhibition enzyme immunoassay. Results: There was a high significant increase in RBP4 and visfatin levels (58.43±18.04) and (7.79±2.26) ng/ml respectively in obese NAFLD (G1) according to the control (G1) (10.07±3.40) and (2.37±0.20) ng/ml. While there was a significant decrease in vitamin A level (28.24 ±1.39) ng/ml in G1 compared to G3.
(54.65±3.70) ng/ml. A non significant increase in RBP4, visfatin levels and vitamin A levels (58.43±18.04), (7.79±2.26) and (28.24 ±1.39) ng/ml respectively for obese NAFLD (G1) according to non obese NAFLD (G2) (52.4±3.49), (6.88± 0.62) and (28.06±6.95) ng /ml respectively. Conclusion: Serum RBP4, visfatin and vitamin A associated with obese NAFLD Iraqi patients.

Keywords---Non alcoholic, fatty liver disease, RBP4, visfatin, body max index.

Introduction

One of the most common public liver diseases over the world is fatty liver which contain alcoholic and non-alcoholic fatty liver. One-fourth among general population are impact Non-Alcoholic Fatty Liver Disease (NAFLD) in the worldwide (1). NAFLD is known as a commutation of excessive fat (> 5%) as triglycerides form (steatosis) in the liver by excluding alcohol consuming (2). The pathogenesis of NAFLD is known as a multi factorial disease correlated with metabolic dysregulation, unhealthy lifestyle, diets, oxidative stress, inflammation, insulin resistance and altered gut liver axis addition to the genetic factor (3). The increased levels of lipids result changing in lipolysis which liberate free fatty acids and these generate more reactive oxygen species (ROS) in respiratory chain reactions (β-oxidation), causing lipid peroxidation(4, 5).

Retinol binding protein 4 (RBP4) is known as an adipokine and one of lipocalin family, it possess a tertiary structure of protein known as "lipocalin fold"(6). It is mainly synthesized and secreted from the liver and form adipose tissues as secondary secreted in spite of other tissues such as adipose tissue, skeletal muscle, kidney, testis and ovary (7). RBP4 acts as a transporter and specifically bound to retinol (vitamin A /retinol acid (RA)) from liver to others tissues(8). Recently, RBP4 can transport fatty acids and has a significant role in lipid metabolism (6,9). RBP4 has role in production, regulation of cell proliferation and differenation addition to its role in immune system (10). The expression of RBP4 in adipose tissues is regulated by 17-estradiol, therefore the RBP4 levels will be elevated in women with poly cystic ovary syndrome and obesity (11).

Visfatin is an adipocytokine, known also as pre-β cell colony-enhancing factor (PBEF) as well as nicotinamide phosphoribosyl transferase (NAMPT)(12). Visfatin is mainly produced from visceral fat tissue, skeletal muscles as well as liver, and also expressed in other tissues like heart, activated lymphocytes bone marrow, lungs, kidney and placenta (13). Vitamin A absorbed, transported as retinyl esters to the liver then hydrolyzied to the retinol form and storage in hepatic stellate cells (HSCs) after reesterified with triglycerides. Hepatic retinyl esters are hydrolyzed to retinol and binds to its carrier RBP4 then transport to the target tissues (14, 15). This research aimed to find the relation between RBP4, visfatin and vitamin A in obese and non obese NAFLD Iraqi patients.
Materials and Methods

Ninety serum blood samples were collected for the period from November 2021 to March 2022 from the Gastroenterology and Liver Teaching Hospital and Baghdad Hospital Consultation within the Medical City Department. The samples distributed into three groups:

- First group (G1) contained of (34) patients of obese Non- Alcoholic Fatty liver disease (NAFLD), 18(52%) were female and 16(48%) were male patients with BMI (35 kg/m²).
- Second group (G2) contained of (26) non obese NAFLD, 16 (61.53%) of them female and the rest (38.46) male, with BMI (23 kg/m²).
- Third group (G3) control patients contained of (30) patients 12(40%) of them were male and 18 (60%) were female, with BMI (22 kg/m²).

The NAFLD patients complicated with other disease were excluded from this study. Ultrasound was used to confirm the diagnosis of NAFLD by examining the abdomen. Adiponectin and visfatin kits were provided from Elabscince Company, USA. The Sandwich ELISA principle was applied on these kits. Vitamin A was provided from My Bio Sourse Company, USA and the competitive inhibition enzyme immunoassay was utilized. Body mass index (BMI) was measured according to the mathematical equation (BMI= weight (Kg)/High (m²) and the correlation between RBP4 with visifatin and vitamin A was obtained in this study.

Statistical analysis

The data were statically analyzed by variance ANOVA using Computer Windows DESKTOP-83QUV4R Program Microsoft Office Excel 2010. For comparing between obese, and non-obese NAFLD patients with the control group, t-test was used at the probability level (P<0.001).

Results

The data obtained from this study was listed in the table(1). The (mean± SD) value for RBP4 was higher in G1 (58.435±18.043 ng/mL) than G2 (52.4±3.497 ng/mL) and it was five times greater than in G3 (10.07±3.400 ng/mL). The P value of RBP4 of G1 vs. G2 (0.762), G1 vs. G3 (P≤0.001) and G2 vs.G3 (0.053).

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<th>Parameters</th>
<th>Mean ±SD</th>
<th>P value</th>
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<tr>
<td>RBP4 (ng/mL)</td>
<td>G1=No.34</td>
<td>G2=No.26</td>
</tr>
<tr>
<td>RBP4 (ng/mL)</td>
<td>58.43±18.04</td>
<td>52.4±3.49</td>
</tr>
<tr>
<td>Visfatin (ng/mL)</td>
<td>7.79±2.26</td>
<td>6.88± 0.62</td>
</tr>
<tr>
<td>Vitamin A(ng/ml)</td>
<td>28.24 +1.39</td>
<td>28.06+6.95</td>
</tr>
</tbody>
</table>

Table 1
The (mean ± SD) of RBP4, visfatin and vitamin A
G1: Obese nonalcoholic fatty liver disease (NAFLD), G2: Non obese nonalcoholic fatty liver disease, G3: controls.

The results have been showed increase in concentration RBP4 serum in obese NAFLD patients and non obese NAFLD compared to G3: healthy or control group. This finding is agreement with a previous study that showed increased systemic RBP4 concentrations can be seen in conditions of obesity; glucose tolerance, and T2DM. In the liver, RBP4 increases fasting blood glucose by augmenting the action of phosphoenolpyruvate carboxykinase (PEPCK) enzyme increasing hepatic glucose liberation(16). The association of RBP4 levels with NAFLD is still conflicting. Many studies finding correlation between RBP4 with NAFLD in the general population, some of these studies supported that RBP4 levels increased and significantly correlated with NAFLD(17-19), while others found that RBP4 independently positively correlated with NAFLD(20,21). Other studied reported that RBP4 levels significantly decreased in NAFLD patient (22).

Other study suggested that visceral obesity might act a role by increasing RBP4 level and some studies were conducted to reduce weight of subjects by bariatric surgery or lifestyle alteration, these results showed that RBP4 level decreased markedly, accompanied by reduction in visceral fat mass(23). The visfatin levels (mean ± SD) were (7.794±2.265), (6.880±0.62) and (2.379±0.205) (ng/mL) for G1, G2 and G3 respectively. The P value of G1 vs. G2 (0.103), G1 vs. G3 0.001 and G2 vs. G3 (0.001). Visfatin level increases through obesity development and correlated with different inflammatory conditions(24, 25). In our study we found serum visfatin increased in G1 and G2 compared with G3. This in agreement with another study that revealed visfatin level was significantly increased in NAFLD patients when compared with control group (26). Previous literature confirmed visfatin level increases with BMI in females with visceral obesity(27), unfortunately, this study did not adjust sex different. Visfatin levels showed increased in plasma overweight and obese. This elevation of visfatin comes from the increases of expression and secretion of pro-inflammatory and adipocytokines(28). George S. Riada and colleagues confirmed that increased serum vasfatin level in patients with NAFLD with subsequent decrease when NASH is diagnosed, so this protein can be used as a noninvasive marker for NAFLD and as an unfavorable prognostic indicator for development of NASH (29).

The values of of vitamin A (mean ±SD) were (28.245±1.399), (28.064±6.950) and (54.65±3.707) (ng/dl) for G1, G2 and G3 respectively. Retinoid is primary storage in the liver, about 80% of the total body retinoid stored as retinyl esters in triglyceride-rich lipid droplets in quiescent hepatic stellate cells (HSCs), specialized mesenchymal cells of the liver (30). The HSCs activated and rapidly loss their vitamin A contents in response to liver injury and this process linked to Chronic liver diseases including NAFLD(31, 32). It is unclear, the level of hepatic retinoid are reduced in high fat diet, NAFLD(33-36), and acute liver injury(37). The level of hepatic retinoid were inversely correlated with hepatic steatosis and NAFLD, this suggesting that ectopic hepatic lipid itself perhaps an early excite hepatic retinoid losing before the onset of HSC activation(38). It was found that reduces retinol combined with elevated level of RBP4 in metabolic syndrome and obesity (14).
Table 2 Listed the level of serum GPT/ALT, GOT/AST and ALP (mean±SD) in obese and non obese NAFLD and control groups.

<table>
<thead>
<tr>
<th>Parameters</th>
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<td></td>
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<td>G2,No.=26</td>
<td>G3,No.=30</td>
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<tr>
<td>ALP (U/L)</td>
<td>95.52±38.88</td>
<td>188.86±36.84</td>
<td>63.56±15.75</td>
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<tr>
<td>SGPT/ALT (U/L)</td>
<td>40.21±17.00</td>
<td>130.97±56.65</td>
<td>27.88±6.88</td>
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<tr>
<td>SGOT/AST (U/L)</td>
<td>40.70±2.44</td>
<td>99.84±34.84</td>
<td>25.44±6.16</td>
<td>0.452</td>
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<td>0.284</td>
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<tr>
<td>AST/ALT</td>
<td>1.012±1.43</td>
<td>0.76±0.61</td>
<td>0.912±0.89</td>
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<td>1.136</td>
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G1: Obese nonalcoholic fatty liver disease (NAFLD), G2: Non obese NAFLD and G3:Control

The values of serum GPT/ALT (Mean±SD) were (40.21±17), (130.97±56.65) and (27.88±6.88) (U/L) for G1, G2 and G3 respectively. The P value were (0.373) for G1 vs. G2, (0.044) for G1vs. G3 and for G2 vs. G3 (0.250). Alanine aminotransferase (ALT) is exists in the cytosol of hepatocytes, its level increased in blood stream during hepatic inflammation and liver injury. ALP consider as standard indicator for liver function assessment and monitoring the patients with liver diseases(39). The obtained data from this study referred to increase in GPT/ALT serum level in G2 compared with G1 and G3. Many studies revealed that ALT level increased with NAFLD (40-41) and the elevation is associated with NASH and advanced fibrosis(42). While other study referred that the patients with NAFLD have normal levels of ALT especially as the disease progresses and more than half of patients with NAFLD have normal liver enzyme concentrations(43,44,45).

The values of serum (GOT/AST) (mean ± SD) were (40.70±24.44), (99.84±34.84) and (25.44±6.16) (U/L) for G1, G2 and G3 respectively and P value was G1vs. G2 (0.452), G1vs. G3 (0.018) and G2 vs. G3 (0.284). The obtained data revealed GOT/AST increased in G2 as compared with G1 and G3. The high ratio of AST/ALT (1.012± 1.437) belong to G1 as compared with G2 and G1 (0.762±0.615) and (0.912±0.896) respectively.

During the last two decades, studies have been using the AST/ALT ratio in the recognition of NAFLD from alcoholic liver disease. The ratio of AST/ALT refers to the ratio between the concentrations of these two enzymes in the blood. The normal AST/ALT should be (<1) and this ratio elevated with hepatocytes injury (AST/ALT > 1) and in patients with NASH, alcoholic liver disease (AST/ALT > 2) and hepatitis C(46). The value of ALP (mean ± SD) was (95.52±38.88), (63.56±15.75) and (188.86±36.84) (U/L) for G1, G2 and G3 respectively and P value was G1vs.G2 (0.001) P value G1vs.G2 (0.109), G1 vs.G3 (0.001) and G2 vs.G3 (0.031). In this study there an increase in ALP level in non obese NAFLD patients this agree with a previous study showed that ALP in non obese NAFLD
patients higher than obese NAFLD patients (47). This increasing may be back to metabolic syndrome is a significant risk factor for NAFLD severity (48,49). Other study showed a slight increasing in Serum ALP level in NAFLD patients (50).

Table (3) illustrated the correlation between RBP4 with visfatin and vitamin A in obese NAFLD Iraqi patients.

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<tr>
<td>Vitamin A</td>
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<td>0.036</td>
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RBP4 gave a high significant positive correlation with visfatin and vitamin A, ($P<0.001$) as in figure (1) and (2).

Figure 1. Correlation between Visfatin and RBP4 in obese NAFLD

Figure 2. Correlation between RBP4 and Vitamin A in obese NAFLD
Table (4) illustrated the correlation between RBP4 with visfatin and vitamin A in non obese NAFLD Iraqi patients.

<table>
<thead>
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<td>RBP4 correlation in non obese NAFLD</td>
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<td>Visfatin</td>
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<tr>
<td>Vitamin A</td>
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<td>-0.291</td>
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A high significant negative correlation was obtained between RBP4 and visfatin and with Vitamin A in non obese NAFLD and (P<0.001) as in figures (3) and respectively (4).

![Correlation RBP4 with Visfatin in non obese NAFLD](image1)

![Correlation of RBP4 with Vitamin A in non obese NAFLD](image2)

In conclusion the high level of serum RBP4, visfatin and vitamin A is associated with obese NAFLD Iraqi patients. A significant correlation was found between RBP4, visfatin and vitamin A in the same patients. This elevation can be predicted...
of NAFLD especially in obese besides routine diagnosis of ultrasound examination.

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