RESEARCH ARTICLE

CORRELATION BETWEEN FATTY BINDING PROTEIN 1 (FABP1) AND DIABETES TYPE 2 (T2DM)

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Manuscript Info

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Manuscript History
Received: 23 April 2021
Final Accepted: 25 May 2021
Published: June 2021

Keywords:-
T2DM, FABP1, Correlation, Novel Biomarker

Abstract

Background: Diabetes mellitus is best described as a condition that is characterized by postprandial hyperglycemia that has two types; diabetes type 1 and type 2. Many patients with type 2 diabetes can be asymptomatic. There are many novel biomarkers for the detection of diabetes type 2, such as FABP1, which is investigated as a marker to detect patients with diabetes type 2.

Aim: The aim of the study was to investigate FABP1 in Patients with diabetes type 2 and to find the correlations between FABP1 and fasting insulin in those patients.

Methods: Samples were collected from 99 diabetic patients and 85 samples of healthy participants as the control group. All participants were subjected to liver enzymes (ALT and AST) lipid profile (triglycerides, HDL, LDL, and cholesterol), T-billirbun, Albumin, Alp, AFP, BMI, S-creatinine, Hb, Fatty acids, F. Insulin, CA19.9, HbA1c, and Fbs that were done by an autoanalyzer. The serum level of fatty acid-binding protein 1 was measured by SunRed human FABP1 Elisa kits.

Results: Data was represented as mean ± standard deviation or median with statistically significant values of ALT, AST, ALP, PT, and INR (at P< 0.05). Findings revealed a significant positive correlation between our marker FABP1 and TG, cholesterol, LDL, Fasting insulin, and CA19.9.

Conclusion: FABP1 can be used as a novel marker to detect patients with diabetes type 2.
Introduction:-
Diabetes mellitus is best described as a condition that is characterized by inappropriate fasting or postprandial hyperglycemia: its metabolic effects, including impaired protein and fat metabolism (Barnett, 2005). The disorder has two main manifestations (ADA, 2010). Type 1 diabetes is normally caused by autoimmune disruption of the pancreatic islets of Langerhans. Antibodies exist in the serum of patients with type 1 diabetes, including insulin itself, to many components of the islets of Langerhans (Loura and McEntyre, 2004). Type 2 diabetes is much more common, accounting for about 90 percent of all cases than type 1 diabetes (Goyal and Jialal, 2019). The dietary guidelines are important for diabetic patients as proper nutrition is a pivotal factor in the practical treatment of type 2 diabetes. The nutrition recommendations help the slowing of diabetes progression (Peckldou et al., 2010). Many diabetic patients with type 2 diabetes are asymptomatic; however, when regular measurements are performed, they show elevated blood glucose levels, they are also diagnosed with type 2 diabetes (ADA, 2004). Biomarker refers specifically to a function that is indicated as a sign of a therapeutic presence of pathogenic processes, natural biological processes, and pharmacological responses (Mayeux, 2004). Biomarkers are including α-hydroxybutyrate, Peroxisome proliferator-activated receptor, Fructosamine, Fetuin-A, Carcinoembryonic antigen, Carbohydrate antigen 199, Glycated Haemoglobin, Fetuin-A and Ferritin, transferrin, and the mammalian FA-binding proteins (FABPs) bind long-chain FA. All FABPs bind both saturated and unsaturated long-chain FA (Richieriet al., 2000). There’re many types of fatty acid-binding proteins such as FABP2, FABP3, FABP4, FABP5, FABP6, FABP7, and FABP1. The other is the carboxylate group interacting with K31 and S56 near the protein surface in the portal region (Wang et al., 2015). The gene ablation of FABP1 was found to affect the high-fat diet, where the gene ablation was found to diminish the effect of a high-fat diet on brain endocannabinoid levels (Martin et al., 2017). FABP1 may be a new diagnostic marker for the diagnosis of liver injury (Akbalet al., 2013) and a marker of diabetic nephropathy (Tsai et al., 2020). The objective of the current study is to investigate FABP1 in Patients with diabetes type 2 and to find the correlations between FABP1 and fasting insulin in these patients.

Subjects and Methods:-
The study was conducted on two groups; 99 diabetic patients as a patient group and 85 healthy participants as the control group in the duration from November 2020 to February 2021. We excluded patients with any infection, including viral hepatitis, autoimmune disease, cardiovascular disease, diabetes-related complications including proliferation retinopathy, autonomic neuropathy, diabetes type 1, pregnancy, alcohol consumption, and cigarette smoking.

A blood sample of 7 ml was drawn from each patient in clean, dry plastic tubes and left for 15 minutes to coagulate at room temperature, then centrifuged at 3000 xg for 15 minutes in a cooling centrifuge; the serum was used for the determination of several laboratory investigations. For the determination of white blood cells, 2 ml of whole blood was collected in a plastic tube containing EDTA as an anticoagulant. For determination of INR, freshly collected blood was taken in a tube containing liquid citrate.

All participants were subjected to liver enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT)), lipid profile (triglycerides, HDL-, Cholesterol), glucose, fasting insulin (for calculation of insulin resistance), gamma-glutamyl transferase (GGT), total bilirubin, creatinine, hemoglobin (Hb), alpha-fetoprotein (AFP), carcinoantigen 19.9 (CA19.9) and anthropometric measures for body mass index (BMI) and all the previous parameters were assayed. Serum level of fatty acid-binding protein 1 was measured by SunRed human FABP1 Elisa kits catalog No. 201-12-2160. The study duration was from November 2020 to February 2021, and informed consent was obtained from each participant before participating in this study.

Statistical analysis:
Analysis of data was performed using SPSS program version 21; qualitative data were represented as numbers and percents, whereas the quantitative data was represented using mean and standard deviation. The positive and negative predictive values, sensitivity, specificity, and the area under the curve were determined by the receiver operating characteristic curve. P-value at ≤0.05 was considered significant.

Results:-
Table (1) showed the comparison between group 1(Diabetic patients) and group (2) control group regarding age, gender, whereas table (2) shows the laboratory investigations of ALT, AST, ALP, INR, albumin, creatinine, glucose, cholesterol, Tg, Alkph, HDL, LDL, fasting insulin, CA19.9, and WBCs. There were significant differences in BMI, gender, ALT, AST, ALP, INR, Creatinine, AFP, WBCs, Pt, HDL, LDL, CA19.9, Tg, Alkaline phosphatase, GGT,
Glucose, fasting insulin, cholesterol, and total bilirubin among diabetic patients when compared to control group, while there were no significant differences regarding albumin and Hb among diabetic patients when compared to control group.

Table 1: Demographic features of diabetic patients and control.

| Characteristics | Group 1 Diabetic patients | Group 2 Control | P-value |
|-----------------|---------------------------|-----------------|---------|
| Age (years)     | 57.92 ± 9.01              | 55.53 ± 9.64    | 0.02*   |
| Gender          |                           |                 |         |
| Male            | 53 (53%)                  | 50 (50%)        | 0.02*   |
| Female          | 47 (47%)                  | 50 (50%)        |         |
| BMI             | 32±3.7                    | 27±2.4          | 0.005   |

Data are presented as either mean ± standard deviation or median with Values statistically significant (at P< 0.05).

Table 2: The laboratory investigations of the two studied groups.

| Variables       | Group 1 Diabetes | Group 2 Control | P-value |
|-----------------|------------------|-----------------|---------|
| ALT (IU)        | 34.97            | 24.8            | 0.45    |
| AST(IU)         | 36.2             | 12.7            | 0.74    |
| ALP (IU)        | 174.95 ± 121.36  | 167.17 ± 121.3  | 0.22    |
| Total Bilirubin mg/dl | 1.00    | 0.9             | 0.57    |
| Albumin g/dl    | 2.62 ± 0.55      | 2.5 ± 0.77      | 0.45    |
| PT (seconds)    | 12.29 ± 4.54     | 18.69 ± 10.9    | 0.56    |
| INR             | 1.46 ± 0.31      | 1.0 ± 0.54      | 0.77    |
| Glucose (mg/dl) | 179±45.5         | 93±28           | 0.001*  |
| Cholesterol (mg/dl) | 198.3 ± 27.8 | 148.1 ± 18.8   | 0.000*  |
| Creatinine mg/dl| 0.96±0.52        | 0.73±0.4        | 0.33    |
| TG (mg/dl)      | 171.1 ± 34.2     | 145 ±21.3       | 0.000*  |
| FABP1 (P mol/L) | 40 ±3.6          | 57±7.8          | 0.007*  |
| HDL (mg/dl)     | 38.4 ± 9.6       | 43.8 ± 9.7      | 0.000*  |
| LDL (mg/dl)     | 130±20.5         | 107.9 ± 10.99   | 0.000*  |
| AFP (ng/ml)     | 4.7 ± 1.7        | 5.96 ± 2        | 0.4     |
| Alkph (U/L)     | 251.8 ± 118      | 193.5 ± 42.1    | 0.000*  |
| GGT (U/L)       | 37±9.9           | 34 ± 9.2        | 0.02*   |
| Fasting-insulin (mg/dl) | 17.5 ± 9.3 | 3.1±1.2        | 0.000*  |
| Hb (g/dl)       | 11.1 ± 1.1       | 11.9 ± 1.7      | 0.000*  |
| WBCS (c/mm)     | 7321±3835        | 7196±2270       | 0.8     |
| CA19.9 (U/ml)   | 44±5.3           | 33±7.3          | 0.04    |

Data are presented as either mean ± standard deviation or median with; ALT: Alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; PT: prothrombin time; INR: international normalized ratio 
*: Values statistically significant (at P< 0.05).

The Receiver Operating characteristic (ROC) curve of FABP1 for early detection of diabetes type 2 revealed that the area under the curve (AUC) was 85.7% (P<0.0001) for FABP1 (figure1). The concentration of FABP1 was associated with T2DM patients, with a sensitivity of 93.3% and specificity of 80%, which means that FABP1 level increase parallels with T2DM patients and can be used as a novel marker to detect T2DM patients (Table2).
Fig1: The Receiver operating characteristic curve.

Table 3: The Receiver operating characteristic curve of FABP1.

| Test          | Cut-off value | Sensitivity% | Specificity% | PPV % | NPV % | AUC % | P-value |
|---------------|---------------|--------------|--------------|-------|-------|-------|---------|
| FABP1(P mol/L)| 9.5           | 93.3         | 80           | 99    | 93.7  | 85.7  | 0.000*  |

PPV=positive predictive value; NPV=negative predictive value; AUC=area under curve*:Values statistically significant (at P< 0.05).

In table (3), the results showed that patients with T2DM have a high level of LDL, cholesterol, CA19.9, and fasting insulin. Results showed that there was a considerable positive correlation between our marker FABP1 and each of TG, cholesterol, LDL, Fasting insulin, and CA19.9, while there is a negative correlation with WBCs.

Table 4: Correlation coefficient of FABP1 and routine Parameters.

| Correlation FABP1 vs | r- value |
|----------------------|----------|
| TG (mg/dl)           | .250*    |
| Cholesterol (mg/dl)  | .232*    |
| HDL (mg/dl)          | 0.178    |
| LDL (mg/dl)          | .105     |
| CA19 9 (U/ml)        | 0.117    |
| FASTING INSULIN (mg/dl) | 0.296** |
| WBCs (c/mm)          | -.283**  |

* Significant correlation, ** high Significant correlation

Discussion:

Fasting hyperglycemia is a characterization of diabetes mellitus and its metabolic effects, including impaired protein and fat metabolism. This disorder has two main manifestations diabetes type 1 and types 2. Type 2 diabetes involves the resistance of the body's cells to insulin's normal effect of driving glucose from the blood into the cells' interiors. Insulin resistance is the medical term for type2 diabetes mellitus disease; as a consequence, the levels of glucose in the blood begin to increase. Biomarkers are classified into two categories: standard and novel biomarkers. In this study, we used one biomarker from the Fatty acid-binding protein family, which is fatty acid-binding protein 1, to detect patients with diabetes type 2.

In our study, we found that the increase in the level of glucose in the blood is mainly responsible for the observed hyperglycemia. Also, there were significant increases in the level of total cholesterol, LDL cholesterol, triglycerides with a significant decrease in HDL cholesterol in the blood among the patient group compared to the control.
Regarding the activities of liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), the increase in their levels among the patient group wasn't significant. However, the increase in liver enzymes is an indicator of hepatocellular injury and is also associated with insulin resistance.

Similar studies showed that type 2 diabetes was associated with a clinical spectrum of liver abnormalities such as increasing levels of GGT, ALT, and ALK in the patients (Balogun, 2008). In addition, the study by (Sanyal et al., 2015) is in agreement with our study results as it has been demonstrated that abnormal liver function (including increased levels of liver enzymes ALT and GGT) was frequently associated with type 2 diabetes mellitus. Our findings are consistent with those of previous studies, which have indicated that diabetes is associated with elevated lipolysis, triglyceride synthesis, and hepatic uptake of free fatty acids, and with an accumulation of hepatic triglycerides due to insulin resistance (Pagano, 2002). In this study, the levels of WBCs and differential leukocyte counts were slightly increased in T2DM patients compared to controls. The study by (Vozarova et al., 2002) is in line with our results as it was reported that there was a relationship between WBC and diabetes mellitus as a result of increased inflammatory mediators. Inflammatory agents, insulin, and human blood components form a critical signal for any abnormalities, resulting in an invasion by foreign agents and/or inflammation (Ohshita et al., 2004).

In agreement with our findings, research conducted by (Anjaneyulu et al., 2004) reported that the serum creatinine in diabetic patients indicates progressive renal damage. In another study, CA19-9 level was significantly higher in patients with diabetes compared with the control group, the CA 19-9 levels in patients with diabetes was 31.2%, and CA 19-9 level was positively associated with age, and duration of diabetes (Kamile et al., 2011), which were similar to our study.

In contrast to our results, a study conducted by (Tsai et al., 2020) showed that FABP1 levels were positively associated with creatinine and negatively associated with albumin and eGFR in patients with T2DM, but our results showed that FABP1 positively correlated with Tg (r=0.25), cholesterol (r=0.232), LDL (r=0.105), fasting insulin (r=0.296) and CA19.9 (r=0.117) and negatively correlated with WBCs (r=-0.283). Furthermore, they also found that a higher level of FABP1 in plasma as well as stages 3 and 4 of CKD classes of NAFLD grade 2 and 3 was remarkably estimated compared to the normal or grade 1 of NAFLD. FABP1 is expressed in both normal and diseased human kidneys, which indicated that FABP1 accurately reflects the severity of diabetic nephropathy and that it may be a suitable biomarker for the early detection of nephropathy in T2DM patients (Ikemori et al., 2009).

Another study has shown that the plasma FABP1 concentration was significantly associated with nephropathy in T2DM patients even after controlling for anthropometric variables, fasting glucose, lipid profile, and smoking status. The increasing level of FABP1 showed a significant linear trend and was independently associated with nephropathy in T2DM patients (Tsai et al., 2020). In another study, the results have shown that FABP1 was positively correlated with insulin resistance in humans (Shi et al., 2012), so the results of the previous study are in agreement with our study results. In this study, the patients with T2DM significantly had higher levels of not only glucose and fasting insulin but also cholesterol, TG, LDL, ALK phosphatase, GGT, CA, and BMI. On the other side, diabetic patients tended to have considerably lower levels of HDL and hemoglobin. FABP1 was significantly sensitive and specific for T2DM at a small cut-off value of 9.5 P mol/L.

**Conclusion:**
FABP1 showed high sensitivity and specificity with perfect positive predictive value and high negative predictive value in the detection of patients with type 2 diabetes mellitus showing that it can be used as a novel biomarker for the detection of type 2 diabetes mellitus.

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