Serum Heart Type Fatty Acid Binding Protein Levels in Prediabetes-An Invaluable Cardiovascular Biomarker

Priyamvadha Ramesh1, Ajay Chauhan2*, Parul Goyal3, Amrinder Singh4, Ayushi Singhal1, Asmita Gupta1

Abstract

Background: The pathophysiological effects of diabetes on the heart and the rest of the cardiovascular system begins much earlier in its precedent stage of prediabetes and one major underlying defect is insulin resistance. Heart-type fatty acid binding protein (H-FABP) is a recently studied molecule inherent to the cardiac myocytes found to rise in both coronary and non-coronary heart diseases. The utility of the molecule in prediabetes and its relationship with insulin resistance is being studied.

Objective: The aim of the study is to compare serum levels of H-FABP in prediabetics and controls and correlate them with Homeostatic model assessment – insulin resistance (HOMA-IR).

Methods: 50 prediabetic patients and 50 age, sex and BMI matched controls were employed in the case control study. Serum fasting and postprandial blood sugars, glycosylated hemoglobin (HbA1c), fasting insulin levels were measured in cases and controls. HOMA-IR index was calculated from fasting glucose and insulin values. Serum H-FABP was measured in both cases and controls using Immunoturbidimetric method with anti- H-FABP coated latex reagent kits. The values were compared between both the groups.

Results: The mean serum fasting insulin level among cases was 12.22mIU/ml and that of the control group was 5.37mIU/ml (p value <0.0001). HOMA-IR mean values were 3.31 ± 1.56 and 1.16 ± 0.44 in cases and controls respectively (p= <0.001). The mean serum levels of H-FABP among cases and controls were 6.38± 2.76ng/ml and 3.24 ± 2.47 ng/ml respectively (p <0.0001). The correlation between the two variables, HOMA-IR and H-FABP was also found to be strongly positive (r=0.675). Linear regression analysis showed that for 1 unit increase in HOMA-IR, H-FABP increased by 1.095 and for 1 unit increase in Fasting insulin, H-FABP increased by 0.038.

Conclusion: Prediabetics have a higher risk of cardiovascular morbidity when compared to normoglycemics with insulin resistance being the single most important contributor. Serum H-FABP levels are elevated in prediabetes representing a marker of subclinical cardiovascular disease (CVD).

Introduction

The Diabetes mellitus spectrum includes a group of disorders with hyperglycemia resulting from defects in either secretion or action of insulin or in some cases, both. Almost always a predecessor of type 2 diabetes (the most common type), prediabetes is a state of intermediate hyperglycemia and comprises of impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). A study from South Asia found that there was an incidence of 29.5 for every 1000 person years for prediabetes and that the conversion degree of prediabetes to diabetes was 58.9%.1

All components of the metabolic syndrome have found to be associated with an increased risk for coronary heart disease (CHD) worldwide but the so called ‘Asian Indian phenotype’ adds to the risk. Similar to diabetic heart disease, prediabetic cardiovascular morbidity is also on the rise. Studies on prediabetes and cardiovascular disease (CVD) show that the estimated relative risk (RR) was 0.97 to 1.30 for IGT and 1.12 to 1.37 for IFG.2

Heart-type fatty acid binding protein (H-FABP) belongs to a group of intracellular lipid chaperones, found abundantly in the cytosol of cardiomyocytes, and released into the bloodstream when the myocardium is injured.3 H-FABP has been studied as a diagnostic marker for acute coronary syndrome (ACS) and in other CVD like cardiomyopathy, heart failure, pulmonary embolism and atherosclerosis. Glatz JF et al also reported that experimentally induced
The study was conducted in the Departments of Medicine and Biochemistry at Post Graduate Institute of Medical Education and Research, Dr. RML Hospital, New Delhi.

Study Design: A case control study.

Sample Size The study group consisted of 50 consecutive patients of prediabetes and 50 control subjects from Medicine OPD, Medicine Wards and Medicine Emergencies of Dr. Ram Manohar Lohia Hospital after fulfilling all inclusion and exclusion criteria and matched for age, sex and ethnicity.

Study period: 1st November 2017 to 31st March 2019

Calculation of Sample Size

Primary Objective

To compare the levels of H-FABP in prediabetics and controls.

To achieve the primary objective, the input for statistical sample size calculation was taken from the study by Basek Karbek et al, 2011.

Patients with impaired glucose tolerance showed a mean (±SD) of 32.5±34.2ng/dl and controls showed a mean ± SD of 16.8±14.9ng/dl.

With the above inputs, we considered a minimum difference of about 50% in H-FABP levels in prediabetics and controls.

With this information, the sample size was calculated as follows.

With an α of 0.05%, β of 0.2%, we get a sample size of 49 on each side.

\[
\begin{align*}
\text{Mean 1: } & 16.8; \text{SD1: } 14.9 \\
\text{Mean 2: } & 25.2 (150\% \text{ of } 16.8) \\
\text{Enrolment ratio of } & 1
\end{align*}
\]

\[
\begin{align*}
(n = \frac{2(\text{SD})^2(Z_{1-\alpha} + Z_{1-\beta})}{(\text{difference})^2}) = \frac{2(14.9)^2(1.96+0.84)}{(8.4)^2} = 49 - 50.
\end{align*}
\]

We require a sample size of 49 on each side (for convenience, 50) to achieve the primary objective of comparing H-FABP between prediabetics and controls.

Inclusion Criteria

- 50 Cases of Prediabetes of age 18-65 years as defined by fasting plasma glucose between 100 to 125 mg/dL OR 2 hour postprandial glucose/2 hour oral glucose tolerance test (OGTT)(after 75 gm of glucose solution ingestion) between 140 to 199 mg/dL OR HbA1c = 5.7-6.4% (American Diabetes Association 2016).
- 50 control subjects, matched for age, gender, ethnicity and body mass index and with fasting blood glucose of less than 100mg/dl and 2 hour postprandial glucose/2 hour OGGT of less than 140mg/dl and with no known co-morbidities as per exclusion criteria.
- An informed bilingual written consent was taken from each of the patient/relatives for inclusion.

Exclusion Criteria

- Known Hypertensives.
- Known Diabetics
- Chronic smokers
- Chronic alcoholics
- Known cases of cerebrovascular accidents or transient ischemic attacks [TIA]
- Known hypothyroid or hyperthyroid patients.
- Known established cases of stroke, angina pectoris, myocardial infarction.
- Known cases of peripheral vascular disease and history of intermittent claudication.
- Patients with history of pulmonary embolism
- Known cases of chronic renal failure.
- Known cases of cardiomyopathy and heart failure.
- Known cases of Systemic Lupus Erythematosus (SLE), Vasculitis, malignancy and connective tissue disorders.
- Known retrovirus positive patients
- Patients on drugs like statins and other anti hyperlipidemic drugs and anti platelet or anti thrombotic drugs.

Methods

All the cases and controls underwent the following examinations and tests:

Clinical Examination

The study participants were called to the Department of Medicine, Dr. RML hospital and asked to fill a pre-determined questionnaire which included baseline data about age, sex, race, ethnicity and family history of diabetes or hypertension.

They then underwent a detailed clinical examination including measurement of height (using stadiometer), weight (using a weight measurement scale) and waist circumference at the upper borders of both hip bones (using a standard measuring tape). Body mass index (BMI) was calculated as weight (in kg) divided by the square of height (in meters). Resting systolic and diastolic blood pressures were recorded twice using an automated sphygmomanometer after a 5-min rest and average was calculated.

Laboratory Investigation

Around 10 mL of fasting blood sample was collected after venipuncture. Samples were taken in EDTA vial for glycated hemoglobin measurements. Plain (Red) vials were used to take samples for serum biochemistry including baseline biochemical parameters and separately for H-FABP.

Investigation done on the patients were

- Fasting plasma glucose,
- Post prandial plasma glucose
- Glycated haemoglobin (HbA1c),
- Fasting serum insulin levels, measured by Chemiluminiscence Immuno Assay (CLIA) on Vitros ECiQ by Orthoclinical Diagnostics.

All of the samples were analyzed on a fully automated clinical chemistry analyzer in the Department of Biochemistry, PGIMER and Dr. RML hospital, New Delhi.

- Samples for H-FABP were
**Table 1: Demographic and anthropometric characteristics among cases and controls**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases (n=50)</th>
<th>Controls (n=50)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE (mean ± SD)</td>
<td>35.96 ± 4.48</td>
<td>35.94 ± 4.27</td>
<td>0.982</td>
</tr>
<tr>
<td>SEX (%)</td>
<td>Males 48(24)</td>
<td>54(27)</td>
<td>0.548</td>
</tr>
<tr>
<td></td>
<td>Females 52(26)</td>
<td>46(23)</td>
<td></td>
</tr>
<tr>
<td>BMI (mean ± SD)</td>
<td>24.07 ± 2.71</td>
<td>24.08 ± 2.7</td>
<td>0.99</td>
</tr>
<tr>
<td>Waist circumference (mean ± SD)</td>
<td>82.02 ± 9.22</td>
<td>80.11 ± 13.03</td>
<td>0.775</td>
</tr>
<tr>
<td>Systolic blood pressure (mean ± SD)</td>
<td>116.56±17.08</td>
<td>114.8±9.00</td>
<td>0.521</td>
</tr>
<tr>
<td>Diastolic blood pressure (mean ± SD)</td>
<td>73.76±4.25</td>
<td>73.52±5.68</td>
<td>0.811</td>
</tr>
</tbody>
</table>

**Table 2: Biochemical parameters among cases and controls**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases (n=50)</th>
<th>Controls (n=50)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood sugar</td>
<td>110.86±9.79</td>
<td>86.68±7.14</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Postprandial blood sugar</td>
<td>161.3±21.97</td>
<td>119.6±12.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HbA1c</td>
<td>6±0.21</td>
<td>4.9±0.46</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting insulin levels</td>
<td>12.2±5.42</td>
<td>5.37±1.95</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HOMA-IR index</td>
<td>3.31±1.56</td>
<td>1.16±0.44</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum H-FABP</td>
<td>6.38±0.76</td>
<td>3.24±0.47</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The basal state insulin resistance of the individual was calculated using the HOMA-IR (homeostatic model assessment of insulin resistance) using the formula:

\[ \text{HOMA-IR} = \frac{\text{fasting plasma glucose (mmol/l)} \times \text{fasting serum insulin (mIU/ml)}}{22.5} \]

**Serum Heart Type Fatty Acid Binding Protein**

Serum H-FABP kits were imported from Randox Laboratories, India. Separate kits for cases and controls along with the buffer agents and anti H-FABP latex coated reagents were used. Catalogue number: FB 4025 and FB 4026. The stored serum samples were analysed as a whole by immunoturbidimetric method.

**Principle of the Test**

The samples were assayed on the principle of immunoturbidimetry with the help of a H-FABP calibrator series. The samples were allowed to react with a buffer and anti H-FABP coated latex reagents and the formation of antigen-antibody complex during the reaction resulted in an increase in turbidity, the extent of which was measured as the amount of light absorbed at 700nm. By constructing a standard curve from the absorbance of the standards, H-FABP concentration in the sample was measured.

Measuring range of the kits was 0.747 – 120 ng/ml.

**Statistical Analysis**

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected then non parametric test was used.

Quantitative variables were compared using Independent t test/ Mann-Whitney test (when the data sets were not normally distributed) between the two groups. Qualitative variables were correlated using Chi-Square test/Fisher’s Exact test. Spearman rank correlation coefficient was used to find out the correlation of various parameters with each other. Univariate linear regression was used to find out the cause and effect relationship between various parameters. A p value of <0.05 was considered statistically significant. The data analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

**Results**

The aim of our study was to assess the serum levels of the molecule H-FABP in patients with prediabetes and compare with the same in normoglycemics. It was a case-control study and after calculating the sample size (50) as per statistical analysis, 50 cases and 50 controls were enrolled. Matching with respect to age, sex, BMI, blood pressure and BMI was ensured. The following observations were made (Tables 1, 2).

We infer that for 1 unit increase in HOMA-IR, H-FABP increases by 0.038. For 1 unit increase in fasting insulin, H-FABP increases by 0.038.
The mean serum fasting insulin level among cases was 12.22 mIU/ml and that of the control group was 5.37 mIU/ml (p value <0.0001) (Table 1). Among the cases, 72% of the total number were found to have a serum fasting insulin level of more than or equal to 9 mIU/ml whereas, only 4% of the controls were found to have so (Figure 2). HOMA-IR index in our study showed mean values of 3.31 ± 1.56 and 1.16 ± 0.44 in cases and controls respectively and the difference, statistically significant (p <0.001). Moreover, around 78% of prediabetics and only 4% of controls had an absolute HOMA-IR index of >or equal to 2(p<0.0001). The mean serum levels of H-FABP among cases and controls were 6.38± 2.76ng/ml and 3.24 ± 2.47 ng/ml respectively and the difference in means, statistically significant (p <0.0001) (Table 5). The correlation between the two variables, HOMA-IR and H-FABP was found to be strongly positive with a correlation coefficient of 0.675 (Figure 3). Linear regression analysis showed that for 1 unit increase in HOMA-IR, H-FABP increases by 1.095 and for 1 unit increase in Fasting insulin, H-FABP increases by 0.038 (Table 6).

**Discussion**

The study shows evidence of increased levels of H-FABP in prediabetics compared to normal subjects. The levels also positively correlated with HOMA-IR indices, a measure of insulin resistance. This signifies an alteration in the normal cardiovascular function in such patients as noticed by the increase in the levels of the molecule in various coronary and non coronary heart diseases and metabolic conditions, hence proving as a cardiac biomarker of diagnostic and prognostic significance for the hyperglycemia associated cardiovascular disease.

It was studied that insulin sensitivity, rather than plasma insulin levels, is associated with early atherosclerosis in diabetes and prediabetes. Studies report a link between the molecular pathways of insulin signaling and inflammation. The progression of IR to diabetes parallels the progression of endothelial dysfunction to atherosclerosis. The downregulation of the antiatherogenic phosphatidylinositol-3-kinase-mediated insulin receptor-signaling pathway, and maintained activity of the proatherogenic mitogen-activated protein kinase pathway in insulin-resistant states, leads to accelerated atherosclerosis.

Factors like increased oxidative stress, coagulability, endothelial dysfunction, mitochondrial dysfunction are found associated with insulin resistance in diabetic humans and animals and are supposedly due to the metabolism of excess glucose and fatty acids in the hyperglycemic state contributing to the development of chronic inflammation and CVD.

H-FABP is a recently detected biomarker found to be released into the bloodstream in cases of myocardial injury, as researched in multiple studies. Okamoto et al and Azzazy HM et al proposed that H-FABP was an excellent biochemical marker for the diagnosis of acute Myocardial Infarction (MI) in the early phase and also in predicting further cardiac events in such patients. Farooq Ghani et al suggested that the magnitude of the increase in plasma H-FABP, released also might demonstrate a good correlation with the size of the infarction. Pelsers MM et al noticed that H-FABP proves to be an excellent early cardiac marker in detecting minor myocardial injury in heart failure and unstable angina, apart from ACS. Puls M and his colleagues concluded that H-FABP might be a promising indicator of early right ventricular injury in acute PE.

H-FABP has also been studied in pump-related clinical contexts like dilated and hypertrophic cardiomyopathy, endocardial fibroelastosis, Chronic Obstructive Lung Diseases (COPD) with cor pulmonale and left heart failure, showing significant differences in values pre- and post- treatments, with comparable or probably better results to pro-Brain Natriuretic Peptide (pro-BNP) and troponin T in heart failure syndromes.

Apart from the primary cardiac diseases, studies have been done in various metabolic conditions. Akbal et al researched that serum H-FABP levels were significantly higher in patients with both diabetes and metabolic syndrome thus representing latent cardiac injury. Oktay B and team hypothesised that H-FABP could be used as a marker of cardiac injury in the early asymptomatic period of obstructive sleep apnoea syndrome

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**Table 5: Serum heart type fatty acid binding protein (H-FABP) levels among cases and controls**

<table>
<thead>
<tr>
<th>Serum heart type FABP</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>6.38 ± 2.76</td>
<td>3.24 ± 2.47</td>
</tr>
<tr>
<td>Median</td>
<td>5.93</td>
<td>2.15</td>
</tr>
<tr>
<td>Min-Max</td>
<td>2.92-11.6</td>
<td>0.81-9.26</td>
</tr>
<tr>
<td>Inter quartile Range</td>
<td>3.630 - 8.980</td>
<td>1.710 - 3.120</td>
</tr>
</tbody>
</table>

**Table 6: Linear regression**

<table>
<thead>
<tr>
<th>Serum heart type FABP</th>
<th>Univariate linear regression</th>
<th>Standardized coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unstandardized coefficients</td>
<td>Standardized coefficients</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Std. error</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.095</td>
<td>0.200</td>
</tr>
<tr>
<td>Serum fasting insulin levels</td>
<td>0.038</td>
<td>0.073</td>
</tr>
</tbody>
</table>

![Figure 3: Correlation of H-FABP with HOMA-IR](image)
a relationship bringing ethnicity, geographical distribution into account and find how they affect individually, the values of H-FABP in prediabetes. Follow up studies are also essential to propose the prognostic utility in cardiac diseases in these patients. Studies on the employment of H-FABP as therapeutic targets for metabolic disorders like obesity, diabetes and atherosclerosis are ongoing and these promise future benefit.

Conclusion
Diabetes, or rather the entire spectrum of dysglycemic disorders comprising of Impaired fasting glucose (IFG), Impaired glucose tolerance (IGT) and Diabetes mellitus is associated with CAD, atherosclerosis and systolic and diastolic heart failures and insulin resistance plays a main causative role. H- FABP levels are increased in prediabetics, correlated with insulin resistance and thus predict early subclinical atherosclerosis and heart disease in them. It is imperative to have a high suspicion of such events in any patient with dysglycemia and strategies to devise methods to diagnose the same are warranted in the current scenario.

References