Transthyretin - A Novel Biomarker for Insulin Resistance and Atherosclerosis Risk in Prediabetics

Ayushi Singhal¹, Ajay Chauhan²*, Parul Goyal³, Anil Taneja⁴

Abstract

Objective: The aim of the study was to compare serum levels of Transthyretin in prediabetics and controls and to correlate levels of same with HOMA-IR and mean CIMT.

Method: It was a case control study in which 60 prediabetic patients and 60 controls (age, sex, BMI matched) were employed. Plasma levels of glucose (fasting and postprandial), glycated hemoglobin (HbA1c), and serum levels of insulin (fasting) were measured in both cases and controls. HOMA-IR values in both the groups were calculated using fasting plasma glucose and serum insulin levels. Serum Transthyretin levels were measured using ELISA. The values obtained were compared between cases and controls. In cases, obtained serum levels of Transthyretin were correlated with HOMA-IR values and mean CIMT (measured in cases only using B-mode ultrasonography).

Results: Median (IQR) of serum levels of insulin (fasting in µU/ml) in cases (11.3 (10.175-13.505)) was significantly higher than that of controls (5.73 (4.3-7.1)). HOMA-IR median (IQR) in cases and controls was 3.12 (2.73-3.595) and 1.21 (0.918-1.505) respectively. Median (IQR) for serum levels of Transthyretin was also significantly higher in cases as compared to controls (46.74 (30.43-81.225) and 22.38 (16.628-27.89) respectively). Significant positive correlations were observed between serum levels of Transthyretin with both HOMA-IR and mean CIMT (with correlation coefficients being 0.288 and 0.536 respectively). Univariate linear regression analysis showed that with increase in serum Transthyretin by 1 mg/dl, mean CIMT increases by 0.001 mm.

Conclusion: Individuals with impaired glucose tolerance have been found to have increased risk of atherosclerosis as compared to normoglycemics after excluding other risk factors. Assessment for the risk of same with the help of novel markers can help in diagnosis and intervention at an early stage and thereby preventing risk of further complications.

Introduction

Diabetes Mellitus is a state of hyperglycemia, due to either an inadequate production of insulin or insulin resistance. Its predecessor state where plasma glucose is found to be elevated above normal levels but below that of clinical disease is regarded as prediabetes. As per American diabetes association (ADA), prediabetes encompasses fasting plasma glucose of 100-125 mg/dl, 2-hour postprandial blood glucose of 140-199 mg/dl or HbA1c of 5.7-6.4%.¹

Metabolic changes occurring due to diabetes leading to multiple organ dysfunction is a common knowledge. However, it has been observed that majority of diabetics already have complications when diagnosed. So, following the adage “catch it early” to decrease not only the progression to frank diabetes but also various micro and macrovascular complications and hence the mortality; knowledge about various aspects of the disease in prediabetic stage is essential.² One of the most devastating among all complications is atherosclerosis, characterised by vessel wall narrowing secondary to inflammatory process. It initially remains asymptomatic and silently progresses over decades before actual clinical manifestations (usually occur in middle and late adulthood).³

CVDs, one of the major complications of atherosclerotic process, contributes to world’s largest disease burden and are a leading cause of morbidity and mortality in individuals with diabetes and prediabetes.⁴ In prediabetics, various etiologies are associated with increased risk for CVD including insulin resistance, hyperglycemia, dyslipidemia, hypertension, systemic inflammation, and oxidative stress.⁵ Various novel biomarkers also increase in insulin resistance states and may have a role in these atherosclerotic changes. One among them is Transthyretin (a small globular transport protein with homo-tetrameric structure) synthesized mainly by liver (in serum) and choroid plexus of brain (in cerebrospinal fluid).⁶ Assessment of these atherosclerotic changes in both peripheral and coronary arteries is by measuring intima-media thickness (IMT). Most commonly used among these is Carotid Intima-Media Thickness (CIMT), usually performed by a B-mode ultrasonographic scan as it is a noninvasive, inexpensive, reproducible method.⁷ So identification of raised levels of Transthyretin at initial stages of insulin resistance and its association with CIMT can help in early intervention and thereby decreasing the risk of atherosclerosis progression.

Materials and Methods

The study was conducted after taking ethical clearance from the institutional ethics committee of ABVIMS and Dr.
RML Hospital in the Departments of Medicine, Biochemistry and Radiology.

Study Design: Cross sectional observational study

Study Group: 60 consecutive patients of prediabetes and 60 control subjects from Medicine OPD, wards and Emergencies after fulfilling all inclusion and exclusion criteria were included and matched for age, sex and ethnicity.


Calculation of Sample Size

Primary Objective

To compare serum levels of Transthyretin in prediabetics and controls.

To achieve this, input for statistical sample size calculation was taken from the study by Pandey GK et al (2015). Minimum required sample size with 90% power of study and 5% level of significance is 30 patients in each study group. To reduce margin of error, total sample size taken per group.

Formula used:

\[ N \geq \frac{2(\text{SD})^2 \times (Z_{\alpha} + Z_{\beta})^2}{(\text{mean difference})^2} \]

Where, \( Z_{\alpha} \) is value of Z at two-sided alpha error of 5% and \( Z_{\beta} \) is value of Z at power of 90% and mean difference is difference in mean values of two groups.

Pooled standard deviation (SD) = \( \sqrt{\frac{(S_1^2 + S_2^2)}{2}} \)

Where, \( S_1 \) is SD of 1 group and \( S_2 \) is SD of another group.

Calculations of sample size for Transthyretin

Pooled standard deviation = 200.03

\[ N \geq \frac{2(200.03)^2 \times (1.96 + 1.28)^2}{(169)^2} \]

\[ N \geq 29.41 =30 \text{ (approx.)} \]

Inclusion Criteria

- 60 Cases of Prediabetes of age 30-60 years as defined by fasting plasma glucose between 100 to 125 mg/dl. OR 2-hour postprandial glucose between 140 to 199 mg/dl OR Hba1c =5.7-6.4% (ADA 2016).
- 60 control subjects, matched for age, gender, ethnicity and BMI and with fasting plasma glucose of less than 100mg/dl and 2-hour postprandial glucose of less than 140 mg/dl and HbA1c less than 5.7 with no known co-morbidities as per exclusion criteria.

Exclusion Criteria

- Known hypertensive
- Known diabetics
- Known cases of chronic liver disease
- Known cases of non-alcoholic fatty liver disease
- Known cases of myelodysplastic syndrome
- Patient on maintenance hemodialysis
- Known cases of coronary heart disease
- Known case of cerebrovascular accidents (CVA) or transient ischemic attacks (TIA)
- Pregnant females
- Known cases of inflammatory bowel disease
- Known cases of Alzheimer’s disease
- Known cases of senile systemic amyloidosis.
- Known smokers and alcoholics

Methods

Clinical Examination

- Anthropometric measurement:

The study participants were called to the Department of Medicine and asked to fill a pre-determined questionnaire which included baseline data about age, sex, race, ethnicity and family history of diabetes or hypertension. Then they underwent a detailed clinical examination including measurement of height (using stadiometer), weight (using a weight measurement scale) and waist circumference (using a standard measuring tape). BMI was calculated as weight in kilograms divided by height in meters squared.
- Resting systolic and diastolic blood pressures were recorded twice using an automated sphygmomanometer after a 5-min rest and mean of the two values (both systolic and diastolic) blood pressure was considered.

Laboratory Investigations

- Around 10 ml of fasting blood sample was collected after venepuncture. Samples were taken in EDTA vial for Hba1c measurements and hemogram profile and fluoride vials for plasma insulin. Plain (Red) vials were used to take samples for biochemical profile and separately for Transthyretin.

Investigations done on the patients were:

- Fasting plasma glucose
- 2-hour postprandial plasma glucose
- HbA1c measurement by Immunoturbidimetry method on Vitros dry chemistry analyser by NSGP guidelines.
- Fasting Plasma insulin levels, measured by Chemiluminescence Immuno Assay (CLIA) on Vitros ECiQ by Orthoclinal Diagnostics.

All the samples were analysed on a fully automated clinical chemistry analyser in Department of Biochemistry.

- Samples for serum Transthyretin were centrifuged and stored in aliquots at -20°C in Department of Biochemistry until batch analysed by ELISA (Enzyme linked Immunosorbent Assay).
- Basal insulin resistance of the individual was calculated using HOMA-IR (Homeostatic model assessment of insulin resistance) using formula:

\[ \text{HOMA-IR} = \left( \frac{\text{FPI} \times \text{FPG}}{405} \right) \]

Where, fasting plasma glucose is in mg/dl.

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### Table 1: Demographic and anthropometric characteristics among cases and controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases (n = 60)</th>
<th>Controls (n = 60)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>45.68 ± 8.78</td>
<td>44.48 ± 7.44</td>
<td>0.439</td>
</tr>
<tr>
<td>Sex (%)</td>
<td>0.855</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>46.67% (n= 28)</td>
<td>48.33% (n= 29)</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>53.33% (n= 32)</td>
<td>51.67% (n= 31)</td>
<td></td>
</tr>
<tr>
<td>BMI (mean ± SD)</td>
<td>25.33 ± 2.65</td>
<td>24.93 ± 2.22</td>
<td>0.387</td>
</tr>
<tr>
<td>Waist Circumference (mean ± SD)</td>
<td>84.57 ± 7.3</td>
<td>82.62 ± 8.7</td>
<td>0.287</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mean ± SD)</td>
<td>116.23± 6.66</td>
<td>116.93 ± 8.13</td>
<td>0.541</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mean ± SD)</td>
<td>74.97 ± 5.17</td>
<td>73.43 ± 4.97</td>
<td>0.079</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases (Median (IQR))</th>
<th>Controls (median (IQR))</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose</td>
<td>110 (106-115.25)</td>
<td>86 (79-91.25)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Postprandial plasma glucose</td>
<td>168 (156-184.25)</td>
<td>125 (117-130)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HbA1c</td>
<td>6 (5.9-6.2)</td>
<td>4.9 (4.6-5.125)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting Serum Insulin Levels</td>
<td>11.3(10.175-13.505)</td>
<td>5.73 (4.3-7.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HOMA-IR Index</td>
<td>3.12 (2.73-3.595)</td>
<td>1.21 (0.918-1.505)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum Transthyretin</td>
<td>46.74 (30.43-81.225)</td>
<td>22.38 (16.628-27.89)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

### Table 3: Descriptive statistics of mean carotid intima media thickness (mm) of study subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± Sdev</th>
<th>Median (IQR)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean carotid intima media thickness (mm)</td>
<td>0.61 ± 0.1</td>
<td>0.60(0.5-0.7)</td>
<td>0.4-0.8</td>
</tr>
</tbody>
</table>

### Table 4: Correlation of mean carotid intima media thickness with Transthyretin (mg/dl)

<table>
<thead>
<tr>
<th>Transthyretin (mg/dl)</th>
<th>Mean carotid intima media thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.38 (16.628-27.89)</td>
<td>0.59 (0.4-0.7)</td>
</tr>
<tr>
<td>46.74 (30.43-81.225)</td>
<td>0.70 (0.5-0.8)</td>
</tr>
</tbody>
</table>

### Results

Aim of the study was to assess the serum levels of Transthyretin in patients with prediabetes, compare the same in normoglycemics and to correlate its levels with CIMT and HOMA-IR in prediabetics. It was an observational case-control study and a total of 60 patients and 60 controls were enrolled. Matching with respect to age, sex, blood pressure and BMI was ensured. The following observation was made (Tables 1, 2 and 3).

Median (IQR) of fasting plasma insulin level (µIU/ml) in cases (11.3(10.175-13.505)) was significantly higher as compared to controls (5.73(4.3-7.1)) (Table 2). Fasting plasma insulin levels (µIU/ml) were ≥9 in 83.33% of cases as compared to controls in whom it was 1.67% of the total. HOMA-IR Index showed median (IQR) values of 3.12 (2.73 ± 3.595) in cases and 1.21 (0.918 ± 1.505) in controls with a statistically significant difference (p value <0.0001) (Table 2). Moreover around 91.67% of cases and 1.67 % of controls had HOMA-IR > or equal to 2. Median (IQR) of Transthyretin level (mg/L) in cases was 46.74 (30.43-81.225), which was significantly higher as compared to controls (22.38(16.628-27.89)) (p value <0.0001) (Table 2). Mean value of mean CIMT (mm) of study subjects was 0.61 ± 0.1 with median (IQR) of 0.6(0.5-0.7) (Table3). The correlation of Transthyretin with HOMA-IR Index, Fasting Plasma Insulin and mean CIMT was found to be statistically significant with a correlation coefficient of 0.228, 0.295 and 0.536 respectively and p value of 0.026, 0.023 and <0.0001 respectively (Figure 1 and 2). Univariate linear regression analysis showed that with increase in levels of Transthyretin by 1 mg/L, mean CIMT significantly increased by 0.001 mm (Table 4).
Table 4: Univariate linear regression to find effect of serum Transthyretin (mg/dL) levels on mean CIMT (mm) and fasting plasma insulin

<table>
<thead>
<tr>
<th>TTR (mg/dL)</th>
<th>Mean CIMT (mm)</th>
<th>Fasting plasma insulin level (µIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta coefficient</td>
<td>0.0001</td>
<td>0.009</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.0001</td>
<td>0.005</td>
</tr>
<tr>
<td>P value</td>
<td>0.004</td>
<td>0.253</td>
</tr>
<tr>
<td>Lower bound (95%)</td>
<td>0.002</td>
<td>0.000</td>
</tr>
<tr>
<td>Upper bound (95%)</td>
<td>0.001</td>
<td>0.018</td>
</tr>
<tr>
<td>Equation</td>
<td>0.563+0.001* Transthyretin (mg/dL)</td>
<td>11.199+0.009*TTR (mg/dL)</td>
</tr>
<tr>
<td>R²</td>
<td>19.92%</td>
<td>6.41%</td>
</tr>
</tbody>
</table>

Discussion

The study showed evidence of increased levels of Transthyretin in prediabetics as compared to controls and a significant positive correlation of Transthyretin with mean CIMT, HOMA-IR Index (marker of insulin resistance) and fasting plasma insulin.

In diabetic and prediabetic individuals, insulin resistance along with impairment of insulin signaling, hyperinsulinemia and hyperglycemia lead to decrease in vascular compliance by increasing glycosylation and oxidation of lipoproteins like LDL and VLDL (Very Low-Density Lipoprotein). The atherosclerotic vascular changes are characterized by arterial wall lesion, endothelial dysfunction and leads to vessel wall hypertrophy which later contributes to increased risk of strokes, MI and TIA.

Risk factors leading to prediabetic state are often associated with increased expression of inflammatory cytokines and also infiltration of immune cells in adipocytes which leads to an insulin resistance state and problems linked with this state like dyslipidemia, hypertension, hypercoagulability and atherosclerosis. By exerting inflammatory process RBP4 (novel adipokine) and its binding protein Transthyretin exert their effect in the pathogenesis of insulin resistance, atherosclerosis and thus CVD.

Till date only few studies suggest role of Transthyretin in insulin resistance states and as a predictor of assessing atherosclerosis risk in individuals with insulin resistance. A very similar observation as in our study was done by Pandey GK et al (2015) where they reported significantly raised circulatory levels of TTR (µg/ml) in T2DM (832 ± 310); followed by those with IGT (TTR: 720 ± 214) as compared to those with NGT (TTR: 551 ± 185; P <0.001). Although unlike our study they didn’t observe any significant correlation of Transthyretin levels with insulin resistance. Although Kwanbunjan K et al found a positive association of RBP4 and Transthyretin levels with insulin resistance, concluding their role in stroke and heart disease.

Conclusion

- Transthyretin has a role in atherosclerotic process through its influence on insulin sensitivity and lipid metabolism. It can be considered as a surrogate marker of predicting early atherosclerosis and thereby cardiovascular risk and also helps in early intervention and preventing further complications. It can also improve the predictive value to diagnose early atherosclerosis when combined with other markers. As Transthyretin levels positively correlate with mean CIMT, so it can be used in early detection and intervention of vascular complication.

References