Study of some inflammatory proteins and autoantibodies in diabetes mellitus type II patients in Baghdad.

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ABSTRACT

Present study was designed to evaluate the prevalence of several markers include islet cell antibodies and insulin autoantibodies along with some inflammatory sensitive proteins like Ceruleplasmin and Transferrin in type II diabetes mellitus of recent onset disease in an Iraqi population. A total of 45 patients with type II diabetes mellitus were studied as well as 30 control healthy individuals. The results show a significant increasing serum ceruloplasmin level with non-significant decreasing level of Transferrin in patient group in comparison with control. The same patients show a positive result for autoantibodies that may refer to inflammatory aspects of disease associated with autoimmunity markers.

Key word: sensitive inflammatory protein, Ceruloplasmin, Transferrin, autoantibody, islet cell antibodies, insulin auto antibodies, diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) is heterogeneous group of disorders connected with raised plasma glucose concentration and disturbance of glucose metabolism. The world health organization (WHO) considers DM type II is the most common type found in about 90% of those with D.M (1). It is common disease affecting over 124 million individuals worldwide, most of them are usually older at the onset of disease, who develops disease after 40 years of their age (2). Diabetes mellitus considers as inflammatory disease implicating chronic subclinical inflammation as a factor in the pathophysiology of diabetes (3). Chronic elevated glucose level in DM increases monocyte adhesion to aortic endothelial cells, which is mediated primarily through induction of interleukin -8 (IL-8) (4), and other inflammatory markers that could help in predicting type II diabetes – associated with immuno-inflammatory manifestations characterize the micro and macro vascular disease complications, particularly for high risk populations (2).

Inflammation is associated with increasing level of sensitive inflammatory proteins (SIPs) (1). Increased level of (SIPs) were found in patients with DM type II (5), like C-reactive protein, ceruloplasmin
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...fibrinogen, albumin and transferrin. Ceruloplasmin (Cp) is an abundant 72 sera glycoprotein which contains more than 95% of the copper present in human Plasma (1). It is synthesized mainly in hepatocytes with six atoms of copper incorporated prior to secretion (6), it is secreted into the plasma as an α2-glycoprotein (7). Although it is exerted as copper transporter, Cp may increase in serum protein type II diabetes (8). It is increased level may cause an early progression of atherosclerosis (2,4), therefore it is necessary to clarify the effect of serum Cp level in DM patients.

Transferrin (TF) is a plasma protein that transports iron through the blood to the liver and bone marrow. The gene for TF is in chromosome band 3q21. It is a single polypeptide chain with carbohydrate moieties in the c-terminal and two homologous domains each containing an iron binding Fe3+ site (9). It is synthesized almost exclusively in the hepatocytes, with lesser amount in choroids plexus of the brain (6).

A strong evidence providing in 1974 about the autoimmune nature of type 1 diabetes that was the discovery of islet cell antibodies which can causes B-cells lesion and destruction leads to insulin deficiency and produce autoantibodies in the circulation, like autoantibodies to islet cell cytoplasm (ICA) (10). Because there are no reliable markers for type II diabetes. The absence of markers or manifestations of type I diabetes is often taken as indicating type II diabetes. Adult patients with DM type II may progress a slowly failure of B-Lymphocytes (11). Some patients show an autoimmune nature of the disease, these patients showed a positive test for pancreatic autoantibodies like antibodies direct against cell islet antigen (ICA). A previous study of B-cells function showed that only ICA mostly develop B-cells failure after five years even though it may take up to 12 years until B-cells failure occur in some patients, impairment in the B-cells response to intravenous glucose and glucagon could be used to diagnosis of diabetes (10). While another report showed that needs only three years to destroy B-cells (11).

The aim of present study is to evaluate some sensitive inflammatory protein in the serum of elderly patients with type II diabetes mellitus to clarify its role in the pathogenesis of type II diabetes and their relationship with autoantibodies ICA and IAA in Iraqi patients.

MATERIALS AND METHODS

Forty five (45) patients with previously diagnosed diabetes mellitus were examined by the physicians in Ibn Al-Nafis Teaching hospital but patients with a history of chronic inflammatory disease, smokers, alcoholics, women with hormonal treatment were not involved in this study. The other group consist of thirty (30) healthy
Iraqi individuals were age, sex and ethnic matching with. Age, weight and height were recorded by a questionnaire, body mass index (BMI) was calculated as weight divided by height squared (Kg/m²) and Central obesity was measured with the subject standing midway between the lower rib margin and iliac crest where's the hip was measured at the level of great Trochanters. Blood sample was collected as a fasting blood sample.

The laboratory investigation includes: sensitive inflammatory proteins (SIPs), both of serum ceruloplasmin (Cp) and transferrin (TF) levels estimated by signal radial immunodiffusion (SRID) plates for accurate quantitative determination of proteins in human serum (Biomaghreb-Tunisia). Using specific endplate, with incubation for 48 hr. at 23°C in case of Cp and TF the concentration of Cp and TF were determined from the standard curve (reference Cp and TF concentration, versus square of ring diameter) and expressed as:
Normal value for Cp g/L {0.19 - 0.57}
Normal value for TF g/L {2.1 - 4.3}.

Auto antibodies includes: Islet cell antibody test (ICA) and Insulin autoantibody, both of them were estimated by the indirect immunofluorescence technique (I.I.F). Frozen sections of pancreas were incubated with diluted patients of pancreas serum sample, so the circulating autoantibody in the serum will binding with specific antigens in sections then form stable antigen–antibody complex in the presence of specific antibodies, after washing the sections. The substrate will be incubated with fluorescein conjugated anti-human globulin reagent to give the specific antibody which emits in the fluorescent microscope. The staining will show slightly stain background with positive ICA result, while it will show bright with positive IAA result. The data analysis were done by using the student t test and Chi-square test.

RESULTS AND DISCUSSION

Table 1 shows the mean level of characteristics of study groups, the mean age. Patient group illustrated non-significant decreased in BMI in comparison to healthy control, while patient group recorded significant increase in the Central obesity compared to control.

Table 2 illustrates the value of sensitive inflammatory proteins (SIP) as mean ±SD. Patient group had significant increase of Cp value compared with control while patient group showed non-significant decreasing mean of TF value compared to control.

Table 3 shows distribution of study groups according to the immunological findings of autoantibody. The two kinds of serum
autoantibodies against pancreatic islet cells were found in patients serum and control without significant differences according to Chi-Squire test, moreover there was coexisting of two autoantibody at the same person.

Table -1: Study subjects characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients group</th>
<th>Control group</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>55.7 ± 12.2</td>
<td>46.43 ± 7.53</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.89 ± 4.1</td>
<td>29.57 ± 4.28</td>
<td>0.68</td>
</tr>
<tr>
<td>Central obesity (cm)</td>
<td>106.2 ± 21.1</td>
<td>96.3 ± 18.2</td>
<td>2.16*</td>
</tr>
</tbody>
</table>

*Significant differences P≤0.05

Table -2: Statistical analysis among study group according to the concentration of sensitive inflammatory proteins (g/L)

<table>
<thead>
<tr>
<th>SIP</th>
<th>Patient group</th>
<th>Control group</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceruloplasmin</td>
<td>0.449 ± 0.097</td>
<td>0.377 ± 0.060</td>
<td>4.0*</td>
</tr>
<tr>
<td>Transferrin</td>
<td>3.264 ± 0.758</td>
<td>3.539 ± 0.718</td>
<td>1.58</td>
</tr>
</tbody>
</table>

*Significant differences P≤0.05

Table -3: Distribution of study groups according to the results of autoantibodies

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>ICA</th>
<th>IAA</th>
<th>Coexistence of two autoantibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Patient</td>
<td>45</td>
<td>8</td>
<td>17.7</td>
<td>37</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>3</td>
<td>10</td>
<td>27</td>
</tr>
</tbody>
</table>

X² = 0.870  df=1  P≥0.05 (NS) (between patient and control group for ICA test)
X² = 1.480  df=1  P≥0.05 (NS) (between patient and control group for IAA test)

Results shows significant increasing serum Ceruloplasmin level in patient with diabetic type II, this result supports previous finding that hyperglycemia could be a cause of increasing serum Cp level in DM patients (7, 8, 12). The increasing may related to the role of inflammatory sensitive protein which may synthesized by hepatocytes in response to tissue damage and inflammation (4, 6), at the same time, Cp is an important intravascular antioxidant factor and it protects tunica intima against free radical injury (6). In addition, the function of Cp as a scavenger, may be related to the increasing serum Cp level in a patient of diabetes mellitus (8, 13). The present clinical finding
supports previous reports (6,13,14), that an increased level of Cp may associate with other risk factors when they observed generally higher Cp Level in smokers serum in comparison with non-smokers patients with DM type II.

In another view present data record an increasing mean of Cp with increasing mean of Central obesity in patients group comparing to control, that may be associated with complication of other risk factors, since about 55% of type II diabetes patients are Obese at diagnosis and chronic obese leads to increase insulin resistance (15). Present patients recorded BMI less than control in contrast with increasing mean of Central obesity, which may associate with DM type II, because adipose tissue especially that around internal organs in the abdomen is a source of several chemical signals to other tissues (hormones and Cytokines) (16). These cytokines stimulate the production of inflammatory sensitive protein like Cp (15).

Present data disagree with a previous study recorded a significant decreasing of serum Cp in DM type II patients (2), that elevated level of Cp could be a risk and predict factor for the complication which will associate with diabetic type II (17), like pathogenesis of atherosclerosis, a common feature of DM type II (3). In spite of the significant increasing level of Cp, there was non-significant decreasing in TF level compared to control. This result disagree with previous report which showed significant decreasing in TF level (6), that may be related with the antioxidant deficiency and excessive peroxide–mediated damage may appear in non-insulin dependent DM (18), which in turn associated with an increase serum Cp level as oxidant scavenger so that Cp Facilitates the incorporation of iron into TF and TF inhibits iron ion-dependent OH formation from H2O2 (6,14). An increased oxidant stress has been implicated in the pathogenesis of DM, which will activate inflammatory cell to release a large amount of inflammation sensitive protein like Cp (7), increasing oxidant stress may consume significant quantities of TF that may explain an increasing Cp level and decreasing TF level in present data, because increasing Cp permits the incorporation of iron into TF (14), that iron release from storage site since hyperglycemia may lead to increase availability of transition metals ions (2). Moreover oxidant stress may associate with increasing production of free radicals or may possibly indicate increasing of glycation of proteins that may damage antioxidant proteins (6). All this conditions must be tightly regulated by proteins that transport, sequester, and mobilize iron from stores and Cp is a highly effective antioxidant that can prevent oxidative damage to lipids, DNA and protein (2,14,18).
Present review agree with previous report that recorded prevalence of ICA auto antibodies in the serum of Iraqi diabetic patients (19). Present study recorded a high positive percentage for both patient and control compared with previous study which recorded 4% for ICA that may relate with aging process of the immune system which may lead to increase the frequency of auto antibodies occurrence (20). Present study recorded high percentage for serum IAA level with DM patient (11.11%) and healthy control (3.33%) that may relate with insulin treatment since insulin treatment can stimulate IAA production (21). In another view, present data agree with a fellow up study which recorded presence of ICA in serum of patients with DM type II and improved that in patient considered type II DM with ICA, beta–cells function progressively decrease after diagnosis within three years, whereas beta–cells function in type II diabetic patients without ICA unchange (11). At the same time, the percentage of positive ICA (17.7%) is higher than IAA (11.11%) that agree with recent study had demonstrated that ICA much more than IAA in adult patients of DM (10).

Furthermore, present data shows coexisting of two types auto antibodies at the same patients, that could be reasonable for Iraqi patients and agree with other reports about other population because approximately 10% of phenotypic type II diabetic patients are positive for at least one of the islet auto antibodies (10). Moreover, coexisting of two types auto antibodies at the same patients means these patients of type II diabetes will progress disease complications more rapidly [10], or the progression to insulin dependence is believed to be more raped than in antibody negative (19).

Present data shows the presence of Cp and IAA in study groups both markers may associate with aging, since serum Cp level increase with aging in normal individual (7), besides auto antibodies can be a marker of subliminal auto aggression process against beta-cells, which is caused by aging or of the instability of the immunological system related to aging or both (19).

In conclusion, the present study emphasizes the need for a long-term follow-up of patients with DM type II, also a prospective cohort studies required to clarify the clinical relevance of SIP serum levels in DM patient and the relation between SIP and auto antibodies because present data had indicated a proportion of autoantibodies and SIP among them. Moreover until now the diagnosis of type II diabetes depends on phenotypic characteristic, that distinction is not always perfect, therefore medical staff need a specific predict factors like the immunological, genetics and functional complexities, so the present data may focus on a new parameters which could be used in future in
diagnostic this type of disease, predicting complication may be associated with progression DM and monitoring the disease.

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