Level of GM-CSF in the Sera of Iraqi Diabetic Patients

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This study reveals that mean serum level of GM-CSF was partially elevated in diabetic cases with and without eye disease, with no detection for it in the sera of control groups.
INTRODUCTION
Diabetes mellitus (DM) is the most common endocrine disease with metabolic, vascular and neuropathic components that are interrelated. The metabolic changes which is characterized by alterations in carbohydrate, fat and protein metabolism secondary to absent or markedly diminished insulin secretion or ineffective insulin action [1,2].

Diabetes mellitus has been known from ancient time and was first reported around 1500 B.C. in the Ebers Papyrus found at Iaxour in Egypt "as a condition causing polyuria" [3]. Diabetes was first described as a disease of thirst by Tchang who observed a patient suffering from this disease; drink ten quarts of water per day with a relative degree of polyuria [4, 5].

Type I or Insulin-Dependent Diabetes Mellitus (IDDM) appears in childhood or in the early ten-age years for this reason; it used to be called Juvenile-onset diabetes.

Type II or Non-Insulin-Dependent Diabetes Mellitus (NIDDM) most often develops in adulthood and used to be called adult-onset diabetes.

Gestational diabetes only appears during pregnancy in women with no previous history of type I or type II diabetes and often goes away after pregnancy [6].

The main symptoms of D.M., glucose level build up in blood and urine, causing excessive urination, thirst, hunger and weight loss [7]. Other symptoms include fatigue, weakness and increased susceptibility of infections, particularly kidney and genitalia [8].

Diabetes mellitus is the seventh leading cause of all deaths and sixth leading cause of all deaths caused by disease.

Non Insulin Dependent Diabetes Mellitus (NIDDM) is more common than IDDM and more frequent exhibits familial aggregation, its pathogenesis is less understood, there are probably many different causes of this form of diabetes with interaction of environmental and genetic factors [9, 7].

Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance, but these two factors (obesity & insulin resistance) alone are insufficient to cause diabetes unless accompanied by impaired β-cell function, which in NIDDM has defect in appropriately recognizing glucose as stimulus to insulin secretion, therefore auto-immune destruction of β-cell does not occur [7]. Thus the pathogenesis of NIDDM are metabolic
disorders that are responsible of immune abnormalities characterized by abnormalities of insulin receptor binding or post receptor blocks in cause the development of this form of diabetes [4, 10].

Finally, factors’ favoring a diagnosis of NIDDM associated with the absence of classical symptoms of diabetes because the hyperglycemia develops gradually occur at older age of onset over 30 years and is frequently associated with obesity which is suspected as an etiologic factor [11, 4, 12].

Cytokines which has been designated to include soluble mediators are synthesized and secreted by leukocytes in extremely low concentration [13]. Most manifest their biological effects through specific receptors, with high binding affinities, expressed at the surface of their target cell [14]. They participate in all phase of immune response, they affect proliferation, differentiation and migration of various cells in immune system and regulate both humoral and cellular immune response [14]. They serve as chemical messengers between the immune system and other systems of the body forming an integrated network that is highly evolved in the regulation of immune responses and even the susceptibility of tumor cells to the action of other cytokines such as IL-6 [15].

Granulocyte/macrophage colony-stimulating factor (GM-CSF) is an acidic glycoprotein that stimulates hematopoiesis in vitro and in vivo [16]. The best known cytokine participates in cellular proliferation and differentiation is (GM-CSF). It was observed that GM-CSF plays a pivotal role in wound healing of rat model which may be useful for creating better wound healing in risky patients such as diabetics [17]. Moreover, it was noticed that this activity related to its ability to activate macrophages to synthesize and secreted H2O2 since failure of Monocytes of trauma patients to convert to Immature Dendritic Cells is related to preferential M-CSF-Driven Macrophage differentiation [18]. Furthermore, studies denoted that GM-CSF concentration was low in DM patients to facilitate wound healing [19].

This study was planned to estimate the level of GM-CSF in the sera of diabetic patients to investigate any correlation between the disease and this cytokine beside its role in the disease.

MATERIALS & METHODS
One hundred sera samples for diabetic patients were studied which include 60 patients with eye disease and 40 without eye disease in comparison with 30 patients control group of
diagnosed eye disease, as well as 30 apparent healthy individuals. Enzyme Linked Immunosorbent Assay (ELISA) technique was used to measure, serum levels of GM-CSF in diabetic patients with & without eye disease in comparison with control groups according to the instruction of Diaclone France Com. All patients have been diagnosed by the consultant committee of the Gastro-Intestinal Tract (GIT) Center during the period between Jan. 2003 and Jul. 2004. All the results have been analyzed statistically using F-test for quantitative data, while for qualitative data, the difference in proportions was tested by using Chi-square ($\chi^2$) test with P value of <0.05 as the level of significance [20].

RESULTS
The results present in this study were based on the analysis of data of one hundred patients with clinical evidence of DM type-II, sixty patients with eye disease as a complication of diabetes, the rest forty have diabetes without complication, control groups which include thirty patients having eye disease as patients control and thirty without diabetes apparent healthy control individuals.

Age and Sex Distribution:
The distribution of patients according to the age groups was listed in the above table (1) which shows that NIDDM with eye disease, 18 patients (30%) are younger than 50 years, while 42 patients (70%) fall in old age group. On the other hand, type II without eye disease patients reveals that the majority of patients [i.e.32 individuals] (80%) are below 50 years and only 8 patients (20%) were ≥50 years. On the contrary, in patient controls group; the majority of patients (60%) that complain of eye disease are those above 50 years.

Table (1): Distribution of diabetic patients according to age & gender in comparison with control groups:

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Patients groups</th>
<th>Control groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type II with Eye</td>
<td>N = 60</td>
<td></td>
</tr>
<tr>
<td>Type II</td>
<td>N = 40</td>
<td></td>
</tr>
<tr>
<td>Patients control</td>
<td>N = 30</td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
control
N = 30
Age
groups
(Years)
No % No % No % No %
<40 6 10.0 12 30.0 3 10.0 6 20.0
40-49 12 20.0 20 50.0 9 30.0 18 60.0
50-59 18 30.0 4 10.0 12 40.0 6 20.0
60- 24 40.0 4 10.0 6 20.0 -- -
Total 60 100% 40 100
% 30 100% 30 100%
Range 36-65 38-68 38-62 38-54
Mean 53.1 50.3 46.3 40.9
SD +8.21 +9.66 +6.45 +6.26
P
(ANOVA) *<0.0001
* = P value for type II with eye disease in comparison with healthy control group.
Results in table (1) indicates that the mean age of type II patients with eye disease (53.10 ± 8.21) was higher than the mean age of type II patients without eye disease (50.3 ± 9.66), as shown in Fig. (2). Moreover there is highly significant difference between the mean of age of patients with eye disease in comparison with that for patient controls (46.3 ± 6.45) [P < 0.0001].
Figure (1): Bar chart showing the age distribution of studied groups.
Study groups
0
5
10
15
20
25
No. of patients
Type II with eye
disease
Type II Patients control Healthy control
<40 40-49 50-59 60-
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The frequency of patients according to gender reveals that there were 36 females (60%) and 24 males (40%) for type II with eye disease, with a female to male ratio 3:2. While type II without eye disease patients include 30 females (75%) and 10 males (25%) with ratio 3:1 as illustrate in Fig (3).
Figure (2): Bar chart showing the gender distribution of studied groups.
Estimation of Serum Level of GM-CSF:
The estimated level of GM-CSF in sera of the study groups, type II with and without eye disease and patients control, was carried out. The results were (1.869 ± 1.29), (0.622 ± 0.336), (0.922 ± 0.724) respectively and healthy control group showed undetectable, as shown in Table (2), Figure (3).

Table (2): The difference in mean serum GM-CSF level (pg/ml) between the study groups.

<table>
<thead>
<tr>
<th>GM-CSF Concentration</th>
<th>DM With Eye disease (40)</th>
<th>DM without eye disease (20)</th>
<th>Patient controls (15)</th>
<th>Healthy Controls (15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range(pg/ml)</td>
<td>0.178-4.622</td>
<td>0.178-1.067</td>
<td>0.100-2.500</td>
<td>Undetectable</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.869 ± 1.29</td>
<td>0.622 ± 0.336</td>
<td>0.922 ± 0.724</td>
<td>0.0</td>
</tr>
<tr>
<td>P (ANOVA)</td>
<td>*&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P value for type II with eye disease in comparison with patients control group.
Healthy control
95% CL se rum GM-CSF level (pg/ml)
GM=CSF

Figure (3): Error bar chart showing the mean with its 95% confidence interval of serum GM-CSF level (pg/ml) in the study groups.

Table (3) shows that the frequency of the GM-CSF levels in sera of studied groups, a level of 2.370 pg/ml was considered as a cutoff value of serum GM-CSF (calculation was carried out taking the mean serum GM-CSF + 2SD of patients control), therefore, any serum GM-CSF higher than this value was considered as elevated level.

Table (3): Frequency of GM-CSF level in sera of the study groups.

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>&lt;2.370</th>
<th>&gt;2.370</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type II with eye disease</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td>Type II</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Patients control</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Healthy control</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* A cutoff value of serum GM-CSF (mean + 2SD of patients control)

**DISCUSSION**

According to the age distribution of patients in comparison with control groups the current study's results agreed with that reported by [21-22] that the age of diabetic mellitus patients type II is considered to be an independent risk factor for eye disease as a complication of diabetes mellitus.

Considering the frequency of gender, the present findings agreed with the fact that women with GDM might develop type II diabetes mellitus later in their life [23].

The results regarding DM complications agreed with that reported by others, that eye disease as a complication occurs at least twice as often in diabetic men and three in diabetic women as in the non diabetic population [24].

These results conflicted with that abroad in which type II with and without complication are higher in men than women in Japan [25]. Perhaps the variations in racial and genetic factors beside the geographical differences are the causes for this controversy.
It was denoted that macrophages produced in the presence of GM-CSF alone have more potent APC function and when activated, have greater cytotoxic activity than those produced with M-CSF. This is related to the deficient action of M-CSF since it reduces MHC protein expression [14]. Granulocyte Monocyte Colony Stimulating Factor also is essential for the generation of dendritic cells from their marrow derived precursor cells, and the local release of this cytokine by activated macrophage, T cells and keratinocytes in the course of an immune response is thought to trigger dendritic cell maturation into functional APCs [26]. Also GM-CSF was reckoned as impairment of the intracellular growth of bacteria by a synergistic action of the GM-CSF triggered release of autocrine TNF- and Nitric Oxide [27]. These facts recruit the current results that GMCSF level elevated in significant manner in DM accompanied eye disease in comparison with control groups. However, its concentration was low in DM cases alone in comparison with those involved both DM and eye infection which may be due to reduced hydrogen peroxide production in Neutrophils from patients with diabetes [28]. Furthermore, other cytokines such as IL-2 may participate in DM induction [29]. This criterion may explain the elevation of GM-CSF in DM patients who are suffering from eye infection. Moreover it was proposed that there are spontaneously occurring neutralizing antibodies for GM-CSF in autoimmune diseases [30]. Perhaps these antibodies may arise in DM and to which attributed decline level of GM-CSF in DM cases in comparison with eye disease alone.

T-cells and Monocytes secreted GM-CSF under certain condition to promote growth of macrophages so; it’s detected in a number of human illnesses not in a healthy one [31, 32]. So, in view of all above facts it is clear that GM-CSF even in small quantities; it plays a major role in the inflammatory process that it may be; in some way; enhance the development of some diseases such as Diabetes Mellitus.

REFERENCES
10. Moustschen MD, Scheen AJ & Lefebrre PJ “Impaired immune response in diabetes mellitus analysis of the factors and mechanisms involved relevance to the increased susceptibility of Level of GM-CSF in the Sera of Iraqi Diabetic Patients
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15. Multiple Myeloma Research Center "Phase II trial to treat Multiple Myeloma patients with cytoxan and Vinceristine after cycling myeloma cells with rHuGMCSF.”


