Study ABO / Rh Systems with IL-18 & IL-33 in Iraqi Patients with Diabetes Mellitus Type II

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Abstract:
Type 2 diabetes mellitus (DM) is a group of metabolic disorder disease. The inflammatory markers act as a new risk factor for development of type 2 diabetes with a possible association with ABO/Rh blood groups. Human ABO genes are located on chromosome 9q34.1-q34.2.

The aim of this study was to investigate the possible association between inflammatory markers, interleukin (IL) -18 and IL-33 in type 2DM and ABO blood groups.

Sixty four patients with newly diagnosed type2 DM and control group consist of twenty healthy Iraqi individual. Laboratory test were include ABO blood groups using standard serological procedures and detection IL-18 and IL-33 in serum by ELISA kits.

The Present data showed a significant increase in the serum level of IL-18 between type 2 DM patients and control, while there was no significant difference in the serum level of IL-33. At the same time both study blood groups O patients & control showed lowest level of serum IL-18, while blood group A with allele A showed less concentration of IL-33 in patients & control. Blood group O showed the highest percentage in patients & control, also Rh positive showed higher percentage.

In conclusion, positive relation between IL-18 concentration and risk of type 2 DM, thus may be a predictor for newly diagnostic diabetic patient, while Serum levels of IL-33 might be a predictor marker of disease progression. No associations were found between ABO & Rh groups with type 2 DM.

Key words: type2 Diabetes mellitus, ABO blood groups, IL-18, IL-33.

Introduction:
The ABO blood groups are defined by the presence of two antigens on red blood cells A and B, while type O cells bear neither antigen. The frequency of the common ABO phenotypes varies among different populations and within human sub groups [1]. Different studies about Iraqi population have been done, in Karbela and Najef the Blood group O was the commonest blood group, followed by A, B and AB respectively , in Babylon the Blood group O was the commonest followed by AB, B and A [2-3], also blood group O recorded the highest frequency in Missan population followed by B , A and AB respectively and the Rh positive recorded the highest rhesus phenotype frequency [4] , moreover Kurdish Iraqi people showed the highest frequency of blood groups O ,followed by A , B , and AB respectively , with More than 91% of the study population was Rh positive.
another Iraqi researchers have been found that blood group O and Rh positive was the most common and blood group AB and Rh negative was the least common among Iraqi sabians.[6]. However, many studies demonstrated the role of ABO blood groups in type2 DM, the A allele (blood types A or AB) were less likely to have type 2 DM than those of type B or O and Rh positive was associated type2DM [7], while blood group B was associated with type2 DM in another report [8].

Diabetes mellitus is a metabolic disorder disease resulting from a defect in insulin secretion, insulin action, or both. It is arises from complex interactions between multiple genetic, environmental factors [9] and immunological abnormalities with increased levels of inflammatory markers like IL-1 family [10], which regulate by an ancient gene family, largely conserved in mammals, and consisting of 11 members (IL-1α , IL-1β , IL-18, IL-33 etc.[11], all IL-1 genes are clustered on human chromosome 2, while IL-33 (IL-1F11) is located on chromosome 9p24.1[12] and IL-18 (IL-1F4) is located on chromosome 11q22.2–q22.3 [13].

Interleukin-18 (IL-18) was first described as interferon-gamma (IFN-γ) inducing factor (IGIF). It is a newly identified cytokine with pro-inflammatory activity [13]. Serum levels of IL-18 might be a predictor of progression of diabetic nephropathy as well as cardiovascular diseases [14].

Interleukin-33 was initially discovered as a nuclear factor abundantly expressed in high level from endothelial venules of lymphoid organs, with increased level in sclerosis and rheumatoid arthritis than in controls [15]. IL-33 exerted protective effects in an animal model of obese diabetic mice reducing adiposity, fasting plasma glucose and improving glucose tolerance and insulin resistance [16]. We have been designed this study to investigate the possible association among two inflammatory markers (IL-18 and IL-33) and ABO / Rh systems in Iraqi patients with type 2 DM.

Materials and Methods:
Sixty four patients with newly diagnosed type2 DM were examined by physicians in NDC (National diabetes Center) /AL-Mustansirya University. Patients with chronic inflammation were not involved in this study. The control group consisted of twenty healthy Iraqi individual were age, sex and ethnic matching with patients group and both of patients and control groups divided into sub groups according to the type of blood groups. Laboratory test were include ABO blood groups using standard serological procedures using the anti-A, anti-B and anti-D Monoclonal (Spin react, Spain) and human ELISA kits for detection of IL-18 (Medical Biological Laboratories company \ Japan) and IL-33(eBioscience An Affymetrix company / North America) in serum.

Statistical analysis was done using SPSS version 14 computer software (statistical package for social sciences). T-test was used to test the significance of difference in means between two groups. Duncan test to test the significance of difference in means between more than two groups. We calculated the allele frequencies ( p, q , and r) for the ABO locus for population, we obtained the adjusted estimates of the allele frequencies and we performed a test of goodness-of-fit (chi-square) based on allele frequencies to check the significant deviation of the studied populations from Hardy-Weinberg equilibrium.
Results and Discussion:

The distribution of phenotype frequency of ABO blood groups in study groups show in table-1, the highest increase percentage of blood group O (39.1%) in patient group than A (21.8%) , B (20.3%) and, AB (18.8%) respectively , but there were no significant differences among them , also the highest increase percentage of blood group O in control group (35%) , than A(30%) , B(25%) , and AB (10%) blood groups respectively , but there were no significant differences among them according to Chi-square test \( p \geq (0.05) \) NS (Total comparison for sub study groups). 

There were no significant differences between patient and control groups for each group of blood type (table 1), which mean there was no association between (O , A , B ,AB) phenotype frequency and type 2 DM . This result agrees with a study about Indian population who found no association between the distribution of the ABO blood types and diabetes [17] , also a study about Iraqi population the researcher has been found  that people with blood group O had higher levels of total cholesterol, glucose and diastolic blood pressure, followed by group A, B then AB [18] especially phenotype O is commonest  among Iraqi patients and normal population . Our data disagree with other Iraqi study demonstrated that blood group B was associated with type 2DM and hypertension, also blood group O positive was associated with hypertension [8].We can noticed that patients and control subjects who were carrying the B allele (20.3+18.8) (25+10) they appeared less percentage so they were less likely to have DM T2 , so the other alleles ( A and O ) could be consider as a risk factor for diabetic, in contrast with previous report have been found that individuals with allele A appear less susceptible to DM T2 [7] .

<table>
<thead>
<tr>
<th>Blood groups</th>
<th>Phenotype frequency</th>
<th>Patients</th>
<th>Control</th>
<th>Total sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No %</td>
<td>No %</td>
<td>No %</td>
<td>No %</td>
</tr>
<tr>
<td>A</td>
<td>14 21.8</td>
<td>6 30</td>
<td>20 23.8</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>13 20.3</td>
<td>5 25</td>
<td>18 21.4</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>12 18.8</td>
<td>2 10</td>
<td>14 16.7</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>25 39.1</td>
<td>7 35</td>
<td>32 38.1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>64 100</td>
<td>20 100</td>
<td>84 100</td>
<td></td>
</tr>
</tbody>
</table>

X²= 4.796 \( \text{df}=3 \) \( p \geq (0.05) \) NS (Total comparison for sub study groups)

The distribution of Rh blood group in study groups show in table -2 , both study groups show an increase in Rh positive phenotype frequency (87.5% , 90%) respectively , but there were no significant differences among them according to Chi-square test \( p \geq (0.05) \), at the same time the total Rh positive phenotype frequency show the highly percentage among (88.1%) study groups.

<table>
<thead>
<tr>
<th>Rh group</th>
<th>Patients</th>
<th>Control</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Rh+</td>
<td>56 87.5</td>
<td>18 90</td>
<td>74 88.1</td>
</tr>
<tr>
<td>Rh-</td>
<td>8 12.5</td>
<td>2 10</td>
<td>10 11.9</td>
</tr>
<tr>
<td>Total</td>
<td>64 100</td>
<td>20 100</td>
<td>84 100</td>
</tr>
</tbody>
</table>

X²= 0.137 \( \text{df}=1 \) \( p \geq (0.05) \) N.S (Total comparison ).

The allele frequency of ABO blood groups in present study show in table-3 . The highest significant frequency is for allele O (0.5644) in patient group than A (0.22097) , B (0.212) respectively , also the highest frequency of allele O in control group (0.5831) than A (0.2242) , B (0.1927) respectively .The highest significant frequency of allele O in study groups than other alleles according to Chi-square was in patients group (X²=9.8525), while there were no significant differences among alleles of control groups , these results show that the ABO blood groups and Rh factors
Table-3 Allele frequency of ABO blood groups in study groups

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>0.22997</td>
<td>0.212</td>
<td>0.5644</td>
<td>0.9986</td>
<td>X²=0.8525 df=3 P=0.1999</td>
</tr>
<tr>
<td>Stander deviation</td>
<td>0.03909</td>
<td>0.03833</td>
<td>0.04777</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.2242</td>
<td>0.1927</td>
<td>0.5831</td>
<td>0.9999</td>
<td>X²=0.6682 df=3 P=0.9954</td>
</tr>
<tr>
<td>Stander deviation</td>
<td>0.07039</td>
<td>0.06586</td>
<td>0.08467</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.2217</td>
<td>0.2069</td>
<td>0.5714</td>
<td>0.9991</td>
<td>X²=3.251 df=3 P=0.0397</td>
</tr>
<tr>
<td>Stander deviation</td>
<td>0.05417</td>
<td>0.03315</td>
<td>0.04161</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant differences p < 0.05*, p < 0.01**, non significant p > 0.05

The distribution of study sub groups of blood groups according to the serum level of IL-18 show in table -4. The highest mean of IL-18 in patients sub group AB (99.01±17.64) compared with other sub groups (A, B, O) (96.52±9.09, 91.49±28.10 , 80.76±7.45) respectively , but there were no significant differences among them , also the highest significant mean of IL-18 in control sub group B (73.08±11.53) compared with two other sub groups (A, O) (50.36±10.93, 49.10±9.00) respectively , but there were no significant differences between B control sub groups and AB control sub group ( 69.05±18.25).At the same time, there were significant differences in mean of IL-18 between patient and control in all sub groups of blood groups. However, a clear notice can establise between IL-18 level and blood groups that patient and control subject showed the highest mean of IL-18 in blood groups AB and B, while lowest mean of IL-18 was in blood group O , this finding may related with polymorphism of genes which are close to ABO blood group gene ,that agree with previous study has been recorded a very strongly associated between serum TNF-alpha levels and blood group O [20], while other immunological factors like Von Willebrand factor level increased thrombotic risk in non O blood subjects’ [21].
were no significant differences in the mean level of IL-33.

From present data, the less concentration for IL-33 showed in patients who were carrying allele A. At the same time the healthy individual who has the phenotype A mostly showed less level of IL-33, this finding may be agree with previous finding about an association between allele A and low level of IL-33, this finding may be agree with previous finding about an association between TNF and ABO systems [20]. Therefore, further genetic studies on molecular level to address these points will allow a better understanding of the association of IL-1 family cytokines with blood groups in Iraqi population.

Table-5 Distribution of study groups according to the serum level of IL-33(pg/ml)

| Characteristic of Inflammatory markers (IL-18 & IL-33) in patients and control show in table 6. The mean of IL-18 was significantly higher in patients group (89.81 ± 7.32) compared to controls group (57.47 ± 5.75). There were no significant differences in mean of IL-33 in patients group compared with controls group. |

The present result about Iraqi diabetic patients from Baghdad demonstrated a significant increased level of IL-18 in patients sera compared with control subjects, that agree with another study about Iraqi Kurdish people, the researcher found that IL-18 was significantly higher in diabetic patients compared with healthy subjects [22], but disagree with another study about Iraqi diabetic patients, that showed Interleukin-18 was non-significant increased in patients sera [23]. One of a foreign study illustrated that, a strong positive association between levels of IL-18 and risk of type 2 DM, which was independent of known risk factors for diabetes [24]. In addition, Serum and urinary IL-18 levels were significantly elevated in Japanese patients with type 2 DM as compared with control subjects [14]. IL-18 is pro-inflammatory cytokine associated with prototypical Th1 responses [11], actually it expression is clearly evident in monocytes, macrophages and dendritic cells, also it contributes to host defense and to inflammation through synergism in a cascade of cytokines associated with innate responses, including IL-12 and IL-15 [25]. Therefore, these data add to the mounting evidence that diabetes may be regarded as a chronic low-grade inflammatory state [10].

In present study IL-33 showed no significant differences between patient and control groups such finding

Table-6. Statistical analysis of inflammatory markers (IL-18 & IL-33) (pg/ml) in the sera of patients & control.

<table>
<thead>
<tr>
<th>inflammatory markers</th>
<th>Patients group Mean ± SE</th>
<th>Control group Mean ± SE</th>
<th>T- test</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-18 (pg/ml)</td>
<td>89.81 ± 7.32</td>
<td>57.47 ± 5.75</td>
<td>2.38</td>
<td>0.01**</td>
</tr>
<tr>
<td>IL-33 (pg/ml)</td>
<td>2.12 ± 0.19</td>
<td>2.09 ± 0.33</td>
<td>1.29</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Significant differences p < 0.05* , p < 0.01**, non significant p > 0.05
agree with a recent finding which was submitted by Ladez [26] who found that serum content of interleukins-33 in patients with chronic and aggressive periodontitis were not significantly different from that of the healthy subjects, while another researcher found that IL-33 levels were elevated in sera and synovial fluid samples from patients with rheumatoid arthritis, and correlated with disease activity [27], another one found increased Expression of IL-33 in Severe Asthma [28] these data presented evidences that significant elevated level of IL-33 associated with long term of disease, while our study showed no significant elevated level of IL-33 that may be associated with an early stage of disease especially present study was about patients with newly diagnosis, at the same time IL-33 unlike the other IL-1 family members induces T helper 2 (Th2) immune responses is thought to have anti-inflammatory properties and essentially drives Th2 responses and active IL-33 may be released during necrosis as an endogenous danger signal or ‘alarmin’[29]. In all endothelial cells and epithelial cells that expressed IL-33 in vivo, the protein accumulated in the nucleus and no evidence for cytoplasmic, membrane or extracellular localization. Therefore, it is not yet clear how IL-33 may be released from the nucleus to exert its cytokine activities towards target cells expressing the ST2 receptor at the same a variability was noted between different cells, between different parts of the tissues and between different individuals, suggesting modulation of IL-33 expression by local environmental cues [30].

The present data concluded that serum level of IL-18 is elevated in newly diagnostic type 2 diabetic patients, so can be used for diagnosis of the disease, while serum level of IL-33 is not elevated but may be used as predictor for disease progression, also the present data showed that patients who were carrying the B allele, they were less susceptible to have DM T2 because individuals with allele B show less both Phenotype and allele frequency, so the other allele (A and O) could be consider as a risk factor for diabetic. These results need further studies to understand the interaction among type 2 DM, immunological and genetic factors about Iraqi population.

References:


23. Al-Shawk, R. S. J. 2010. A Thesis "Study of Some Cytokines Profile in a Sample Of Iraqi Type 2 Diabetic Patients and Their Relation With Obesity"


دراسة نظام مجاميع الدم والعامل ABO و Rh - 18 و العامل البين أبيضاضي - 33 في مرضى عراقيين مصابين بالسكري من النوع الثاني

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قسم طب الأسنان / كلية اليرموك الجامعة

الخلاصة:
داء السكري هو مجموعة من الاضطرابات الإيضية و تتمثل عوامل الالتهاب عوامل خطورة جديدة تساهم في تطور مرض السكري من النوع الثاني مع وجود احتمال علاقة بمجاميع الدم والعامل الريسي وقد هدفت الدراسة إلى اكتشاف احتمالية وجود علاقة بين عوامل الالتهاب مثل العامل البين أبيضاضي 31 والعامل البين أبيضاضي 11 عند مرضى السكري من النوع الثاني وعلاقتها بمجاميع الدم شملت الدراسة 64 مريض تم تشخيص الإصابة لديهم بالسكري من النوع الثاني لأول مرة ومجموعة السيطرة 20 شخص سليم اجريت لهم اختبارات مجاميع الدم والكشف عن وجود مصل العامل البين أبيضاضي – 31 والعامل البين أبيضاضي – 11 باستخدام تقنية ELISA ظهرت البيانات وجود اختراعات معنوية في مستوى مصل العامل البين أبيضاضي – 18 بين مجموعات مرضى السكري من النوع الثاني ومجموعة السيطرة في حين لم تظهر اختلافات معنوية في مستوى مصل العامل البين أبيضاضي – 33 ، في الوقت نفسه ظهرت ارتفاعات مادة الدهون الفرعية O للمرضى والسيطرة أقل تركيز للعامل البين أبيضاضي – 18 ، بينما مجموعة مجاميع الدم الحاوية على الأليل A ظهرت أقل تركيز للعامل البين أبيضاضي – 33 في المرضى والسيطرة وظهورت فصيلة الدم O والérieur O على نسبة لدى المرضى والسيطرة وكذلك العامل الريسي. 
أخيرا وجدت علاقة إيجابية بين مستويات العامل البين أبيضاضي – 18 وخثورة السكري من النوع الثاني والذي قد يعتبر مؤشرا لحدوث المرض في حين أن مستويات مصل العامل البين أبيضاضي – 33 قد تكون مؤشر لتطور المرض. وتشير النتائج إحصائيا إلى عدم وجود علاقة بين مجاميع الدم ومجموعة مرضى السكري من النوع الثاني والسيطرة.