Determination of some hematological profiles, inflammatory markers, and their relation with insulin resistance in anemic Type 2 diabetes mellitus patients

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Abstract

Background: Extreme alterations in hematological values and inflammatory biomarkers affecting dependent variables in clinical tests are associated with type-2 diabetes mellitus and anemia. Objective: To investigate some hematological and inflammatory risk factors and their relation in developing insulin resistance in anemic type-2 diabetes mellitus.

Patients and Methods: This case-control study was conducted in 190 patients and healthy subjects in the hospital of Layla Qasim Center in Erbil city and ethical clearance was obtained from the university ethical review committee. The included subjects were divided into three groups: non-diabetic (control) group, non-anemic type-2 diabetes mellitus, and anemic type-2 diabetes mellitus. Blood samples were analyzed for hematological parameters by Coulter counter analyzer, and biochemical tests included glycated hemoglobin, fasting blood glucose, insulin, and some inflammatory biomarkers by spectrophotometry and standard sandwich enzyme-linked immunoassay. Harvested data were analyzed by the statistical package for social science version 23.

Results: The results showed remarkable alterations in glycated hemoglobin, red and white blood corpuscles, and inflammatory parameters in anemic type-2 diabetes mellitus compared to non-anemic type-2 diabetes mellitus. Inflammatory parameters including interleukin-1 and tumor necrosis factor-alpha exhibited a strong positive correlation with anemic diabetes than non-anemic diabetes.

Conclusion: We concluded that some hematological and biochemical parameters were positively related to developing anemic type-2 diabetes mellitus and can be helpful as diagnosing tools for predicting the progression of anemic type-2 diabetes mellitus.

Keywords: Anemia; Insulin resistance; Inflammatory biomarkers; Type 2 diabetes mellitus
Introduction

For diagnosing anemia or diabetes mellitus (DM), hematological and serological tests are frequently used [1]. The clinical basis for the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) is currently used to estimate insulin sensitivity and pancreatic B-cell function in fasting states from plasma insulin and glucose concentrations [2-4]. Inflammatory states followed by metabolic stress that occur in diabetes cause imbalance in urea and creatinine as an alarm for developing diabetic nephropathy [5]. Under this pathological state, immune responses, characterized by the demonstration of proinflammatory cytokines in circulatory blood further add a list of new candidates that can be used to predict the onset or severity of the disease [6, 7]. Clinical tests so far have poorly indexed new relevant parameters and their association with developing anemic type-2 DM (T2DM). It is well reported that pathophysiological parameters have deteriorated with the severity of anemia, diabetes, and anemic diabetes [8-10], but the tendency of some biochemical or blood parameters to fluctuate in dramatic magnitude with the progression and development of these diseases is still not fully clarified. For instance, the vast majority of the literature body has reported association [11-13], regression, or relationship [7, 14] of some independent outcomes with the disease prevalence, while little has been reported to involve the role of each clinical variable in the disease progression, and more particularly when the gender might cause a remarkable deviation in measurable outputs. The significance of the present study implied in determining which clinically tested biomarkers could be more associated with insulin resistance (IR) and developing anemic T2DM that in turn can be useful as a therapeutic or diagnoses target for anemic T2DM.

Patients and Methods

This case-control study was achieved in the hospital of Layla Qasim Center for chronic diseases in Erbil city from June 2020 to December 2020. The study included 190 subjects of healthy, anemic, and non-anemic T2DM patients attending Layla Qassim Center. All subjects were gender and age-matched persons and were divided into three groups: the first group (50 control as the healthy person (24 males and 26 females) whose mean age range in years (45.32 ± 4.0)), the second group (70 non-anemic T2DM under medical treatment (35 males and 35 females) with average ages (45.8 ± 5.6)), the third group (70 T2DM patients with anemia (35 males and 35 females) under medical treatment with age (47.42 ± 3.5 years). A specially designed questionnaire form was applied and the groups were subjected to fill it. The purpose of the study was explained to them and background information, socio-economic status, medical history, and some anthropometric measurements like weight, height, and body mass index were recorded. Standard current criteria were applied for the diagnosis of anemia and T2DM in differentiating the diabetic types. T2DM individuals of either sex and age group of older than 40 years who attended the center were diagnosed previously as T2DM based on the symptoms of diabetes more than 7 years plus fasting blood glucose above 200mg and HOMA-IR.
more than 2.1. The study was carried out and ethical clearance was obtained by the medical care committee that approved the study.

**Collection of the samples**

The sample size was determined by using G-power software version 3.1, an alpha level of 0.05, the effect size of 1, and the test power of 80% were specified. Samples of venous blood (2.5 to 3 ml) were obtained after overnight fasting during the patient's visit to the center and collected by sterile disposable syringes and transferred into disposable test tubes. Sera were separated by centrifugation at (1200g for 15 minutes). The separated sera were stored at freezing point -80°C (Sony, Ultra-low, Japan) until assay. Coulter counter machine (Coulter counter/Hitachi 211Q/Japan) was used to analyze hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), total WBC count, differential leukocytes count (Neutrophil percentage, eosinophil percentage, basophil percentage, lymphocyte percentage, and monocyte percentage). Plasma levels of glycated hemoglobin (HbA1c), serum levels of fasting blood glucose (FBS), and insulin were estimated by the spectrophotometric method (KENZA 240 TX). IR was calculated by the homeostatic model assessment of the HOMA-IR formula [15]. This assay used the quantitative sandwich immunoassay technique specific for human C-reactive protein (CRP) enzyme-linked immune-sorbent assay (ELISA) Kit (Biotech, Inc., CA) 92106, GUB-ZD003, USA) which is based on ELISA standard sandwich technology. Serum levels of interleukin-6 (IL-6) were determined in (GenWay Biotech Inc Ref: GWB-SRK014, USA) batch using a commercial ELISA kit (DRG Instruments, GWB-SRK014, Marburg, Germany) according to the manufacturer's protocol [16]. Human tumor necrosis factor-alpha (TNFα) ELISA Kit (Biotech, Inc., CA) 92131, GUB-ZD057, USA) was used and based on standard sandwich ELISA technology [16].

**Statistical Analysis**

The harvested data were tested for the normality tests using Shapiro-Wilk and Kolmogorov-Smirnov tests using GraphPad Prism (Version 8.3). All data were expressed as mean ± standard error of means (Mean ± SEM). One-way analysis of variance (ANOVA) and the parametric test of Pearson's correlation coefficient (r) was also used to correlate the measured parameters. Accuracy of each of the IL-6, TNF-α, and CRP levels in non-anemic and anemic T2DM patients for the diagnosis of the disease was presented in terms of sensitivity and specificity by using Receiver Operating Characteristic (ROC) curve which is a graphical display of sensitivity on the y-axis and specificity on the x-axis for varying cut-off points of test values using statistical package for the social science (SPSS) version 23. The area under the curve (AUC) is a useful quantitative measure of accuracy. An area of 1 represents a perfect test; an area of 0.5 represents a worthless test. Generally, ROC curves with an AUC ≤0.75 are not clinically useful and an AUC of 0.97 has a very high clinical value, correlating with likelihood ratios of approximately 10 and 0.1 which show differentiation power and, therefore reliable utility as a diagnostic test [17].

**Results**

In the present study, hematological parameters were measured in anemic and
non-anemic T2DM, as could be seen in Table (1), and after the differences were significantly examined in the analysis of variance (not shown in the results), the statistical outputs made a comparison between all groups using Dunnett and Sidak tests. Considering RBC, Hb, and PCV, the parameters were dampened more significantly (p<0.001) in anemic T2DM than non-anemic T2DM patients (p<0.05) compared to healthy subjects. And even between the two diabetic groups, these parameters were remarkably declined in anemic diabetes than in the non-anemic diabetic group.

WBC indices on the other hand, as could be observed in Table (1), were also affected by anemic diabetes more significantly in WBC, neutrophils, lymphocytes, and monocytes (p<0.001) than non-anemic diabetes.

Moreover, referring to the same Table, HOMA-IR in anemic T2DM remarkably elevated compared to non-anemic T2DM cases (p<0.001) that clarified the interaction impact between anemia and T2DM in progressing the alteration in clinical parameters. Additionally, statistical analysis, as shown in the Table, showed that in anemic T2DM all the relevant diabetic profile tests (insulin, FBS, HbA1c) were elevated significantly (p<0.001) as compared with the non-anemic T2DM group. Interestingly, the inflammatory parameters (TNF-α, IL-6, CRP) in the Table have also been further elevated in anemic T2DM in comparison to non-anemic T2DM subjects (p<0.001), indicating that overall severe glycation of hemoglobin resulting from severe hyperglycemic status might be considered as the real contributors to developing anemic diabetes. In Table (2), based on Pearson correlation coefficient, the blood indices, considering WBC, neutrophils which exhibited a moderate strength correlation with HOMA-IR while monocytes in less strength with weak negative correlation in eosinophils and basophils in anemic T2DM when compared to non-anemic T2DM group.

To investigate these contributions of inflammatory biomarkers in anemic T2DM, we used ROC curve to figure out the sensitivity of predicting anemic diabetes throughout these three highly relevant biomarkers, as shown in Figure (1), the sensitivity of IL-6 and TNF-α was increased in anemic T2DM (AUC=0.994, 0.998) compared to non-anemic group (AUC=0.959, 0.987) respectively. Considering CRP, as shown in Figure (1), and because it exhibited a high correlation coefficient value in both cases, it could not be considered as a parametric variable toward anemic diabetes, besides its significance in labeling the severity of T2DM.
**Table (1):** Some hematological and biochemical values in the control group, anemic and non-anemic T2DM patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control N=50</th>
<th>Non-anemic T2DM N=70</th>
<th>Anemic T2DM N=70</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (cell/mm³) x 10⁶</td>
<td>5.033±0.115</td>
<td>4.500±0.111 * †</td>
<td>3.450±0.156 ***</td>
</tr>
<tr>
<td>Hb (gm/dl)</td>
<td>14.10±0.021</td>
<td>12.801±0.216 * †</td>
<td>7.555±0.172 ***</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>41.264±0.100</td>
<td>39.901±0.718 * †</td>
<td>25.000±0.112 ***</td>
</tr>
<tr>
<td>WBC (cell/mm³) x 10¹</td>
<td>6.616±0.277</td>
<td>8.038±0.277 ** †</td>
<td>11.910±0.247 ***</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>61.630±0.680</td>
<td>65.070±0.782 * †</td>
<td>74.860±0.488 ***</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>2.090±0.181</td>
<td>1.940±0.111</td>
<td>2.049±0.128</td>
</tr>
<tr>
<td>Basophil (%)</td>
<td>0.340 ± 0.071</td>
<td>0.574 ± 0.071</td>
<td>0.361 ± 0.070</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>28.510±0.428</td>
<td>22.790 ± 0.582 *** †</td>
<td>19.17 ± 0.599 ***</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>0.340 ± 0.071</td>
<td>0.574 ± 0.071 * †</td>
<td>6.195 ± 0.395 ***</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>11.025 ± 0.111</td>
<td>32.081 ± 0.121 *** †</td>
<td>45.236 ± 0.051 ***</td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>97.532 ± 0.101</td>
<td>249.000 ± 1.117 *** †</td>
<td>330.141 ± 1.124 ***</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>6.500 ± 0.130</td>
<td>8.199 ± 0.421 *** †</td>
<td>10.900± 0.310 ***</td>
</tr>
<tr>
<td>HOMA-IR (mmole)</td>
<td>0.622±0.219</td>
<td>3.030±0.629 *** †</td>
<td>5.427± 0.286 ***</td>
</tr>
<tr>
<td>TNF-α (ng/ml)</td>
<td>3.555 ± 0.129</td>
<td>5.783 ± 0.188 *** †</td>
<td>7.383 ± 0.321 ***</td>
</tr>
<tr>
<td>IL-6 (ng/ml)</td>
<td>2.735 ± 0.180</td>
<td>4.633 ± 1.143 *** †</td>
<td>5.738 ± 0.199 ***</td>
</tr>
<tr>
<td>CRP (ng/ml)</td>
<td>2.946 ± 0.135</td>
<td>8.218 ± 0.212 *** †</td>
<td>11.69± 0.200 ***</td>
</tr>
</tbody>
</table>

*Data were expressed as Mean±SEM. Statistical differences between non-anemic T2DM, anemic T2DM compared to control subjects were expressed with star sign (* p<0.05, ** p<0.01, *** P<0.001). Statistical differences between non-anemic T2DM and anemic T2DM groups were shown with dagger signs († P<0.001)*

**Table (2):** Correlation between HOMA-IR and some blood cells in non-anemic and anemicT2DM subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>non-anemic T2DM</th>
<th>anemic T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r*</td>
<td>p</td>
</tr>
<tr>
<td>Total WBC</td>
<td>0.475</td>
<td>0.001</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>0.522</td>
<td>0.001</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>-0.212</td>
<td>n.s</td>
</tr>
<tr>
<td>Basophil</td>
<td>0.211</td>
<td>n.s</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>-0.430</td>
<td>0.001</td>
</tr>
<tr>
<td>Monocyte</td>
<td>0.390</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*r represents correlation, p significance levels. The data of the two groups were analyzed by Pearson coefficient test after being tested for the normality test.*
Figure (1): ROC curve shows the sensitivity and specificity of IL-6, TNF-α, and CRP in non-anemic T2DM and anemic T2DM groups

**Discussion**

In this case-control study, we attempted to differentiate hematological and inflammatory markers contribution and the nature of their correlation strength in progressing anemic T2DM representing in IR. It was obvious in our results that hematological parameters were prone to dramatic changes in anemic T2DM possibly because of abolishing insulin signaling negatively downregulated proliferation and maturation of blood cells [18] and seemingly has come consistent with other available evidence in that IR induced hyperglycemia was positively associated with reducing Hb, RBCs and PCV values in many studies in both animal and human models.
[19, 20]. However, based on other findings, diabetic status, even if chronic, was not necessarily affected RBC indices as reported by Agrawal et al., Jaman et al., and Alamri et al. [21-23] It is worth noting that RBC indices have been reported to be increased under chronic hyperglycemic cases resulted from IR [24]. Furthermore, while some blood indices alone seem not to be reliable in predicting diabetic cases, in anemic diabetes, it has been reported that anemic diabetes decreases RBC indices [25].

WBC indices on the other hand, and based on the above-mentioned studies, significantly exhibited different alterations in different studies and models indicating that although T2DM might not precisely have an impact on these cells, however under anemic status these parameters can be used as a reliable mean for predicting the progression and development of anemic T2DM. This increment in WBC indices coexisted with IR might be reasonable from the inflammatory response perspective, as was indicated in a case-control study by Choi et al. [26] who showed that insulin reduced IL-6 mediated inflammatory response and the latter was the trigger for WBC activation and proliferation. Moreover, referring to Table 1, that clarified the interaction impact between anemia and T2DM in progressing alteration in clinical parameters. However, the strength of such correlation between IR and blood cells indices might be seen as less positive as concluded by Biadgo et al [27] in a comparative cross-sectional study.

The inflammatory biomarkers studies in the present study were predicted to be elevated as a result of systematic impacts of IR and anemia [28, 29]. It is generally believed that CRP is a non-specific biomarker that appears in certain pathophysiological conditions as an alarm for local, or more widely, systematic inflammatory stress [7, 30]. However, as a causative correlation to IR, some studies have directly implemented CRP in developing IR [30]. Additionally, evidence has been reported on TNF-α implementation in progressing IR and T2DM [31-33]. Moreover, growing evidence has also been documented on molecular changes in hepatocytes affected by IL-6 under inflammatory stress that might promote IR [5, 15, 34]. Taking these findings into consideration besides the evidence highlighted in our results, it seemed to be reasonable to use some hematological parameters for determining the progression and severity of anemic T2DM which uncovers our claim in existing such association between inflammatory and hematological indices and development of anemic T2DM.

Conclusions

Even though some hematological and biochemical biomarkers have been recently known to have appeared in anemic diabetes, however, the strength of such sort of association was poorly reported. In our study, IL-6, TNF-α, HbA1C, HOMA-IR, and total WBC, at least, are the most associated parameters in diagnosing anemic T2DM which probably can be exploited in predicting and treatment of anemic diabetes.

Acknowledgments

We appreciate all the participants of patients and healthy people for their great cooperation in the study, and great thanks to laboratory assistants for their facilitating and help in the hospital.
Recommendations

We recommend researches to be done in both human and animal models for further understanding the nature of deviation in clinical variables and their predictions and risk associations in developing anemic diabete.

Source of funding: This research was funded by ourselves and there is no other funding cover this study or manuscript preparation and publication.

Ethical clearance: The study was carried out and ethical clearance was obtained by the medical care committee of the College of Dentistry-Hawler Medical University.

Conflict of interest: Nill

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تشخيص بعض الدلائل الدموية والعلامات الالتهابية وعلاقتها بمقاومة الأنسولين في النوع الثاني لمرضى السكري

خريمان سيد ابراهيم 1، د.زيان شيرزاد حيدر 2، و.د.حامد حسن احمد 3

الملخص

خلفية الدراسة: ترتبط التغييرات المفروضة في الفحوصات الدموية والعلامات الالتهابية التي تؤثر على المتغيرات التابعة في الاختبارات السريرية بداء السكري من النوع 2 وفقر الدم.

أهداف الدراسة: لتقسيم بعض عوامل الخطر المتعلقة بأمراض الدم والالتهابات وعلاقتها بمقاومة الأنسولين في داء السكري من النوع الثاني من فقر الدم.

المريض والطاقب: أجريت هذه الدراسة على 190 مرضي وأصحاء في مستشفى مركز ليلى قاسم في مدينة أربيل وتم الحصول على الموافقة الرسمية من لجنة المراجعة لأخلاقيات البحث العلمي بالجامعة. تم تقسيم الأشخاص المشمولين إلى ثلاث مجموعات: مجموعة غير مصابة بمرض السكري (مجموعة التحكم) ، ومرض السكري من النوع 2 غير المصاب بفقر الدم ، ومرض السكري من النوع 2 مصاب بفقر الدم. تم تحليل عينات الدم من أجل العلامات الدموية بواسطة محلل كولتر ، وشملت الاختبارات الكيميائية الهيموجلوبين السكري ، جلوكوز الدم ، والأنسولين ، وبعض المؤشرات الحيوية الالتهابية عن طريق القياس الطيفي والإيلازا. تم تحليل البيانات التي تم جمعها بواسطة البرنامج SPSS 23.

النتائج: أظهرت النتائج تغيرات ملحوظة في الهيموجلوبين السكري ، وكريات الدم الحمراء والبيضاء، ومعايير الالتهاب في داء السكري من النوع الثاني المصاب بفقر الدم مقارنة بداء السكري من النوع 2 غير المصاب بفقر الدم. أظهرت العوامل الالتهابية بما في ذلك الالتهاب في الدم مقارنة بمرض السكري من فقر الدم مرتبطًا بمرض السكري غير المصاب بفقر الدم.

الاستنتاجات: خلصنا إلى أن بعض المتغيرات الدموية والكيميائية الحيوية كانت مرتبطة بشكل إيجابي بتطور داء السكري من النوع 2 فقر الدم ويمكن أن تكون مفيدة كأدوات تشخيص لتنبؤ بتطور داء السكري من النوع 2 المصاب بفقر الدم.

الكلمات المفتاحية: فقر الدم؛ مقاومة الأنسولين؛ المؤشرات الحيوية الالتهابية داء السكري من النوع 2

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تاريخ استلام البحث: 3 تشرين الثاني 2021
تاريخ قبول البحث: 28 كانون الأول 2021

قسم العلوم الأساسية - وحدة الأحياء الدقيقة - كلية الطب - جامعة هولير الطبية - أربيل ، العراق

العنوان: تشخيص بعض الدلائل الدموية والعلامات الالتهابية وعلاقتها بمقاومة الأنسولين في النوع الثاني لمرضى السكري

التأكد من أن النص باللغة العربية هو الصحيح وألا تحدث أي خروقات لحقوق النشر أو قواعد الاستخدام.