ASSESSMENT OF SERUM HIGH MOBILITY GROUP BOX -1 LEVEL AS PREDICTOR OF DIABETIC PERIPHERAL NEUROPATHY

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Abstract

Background: High mobility group B1 is associated with central ischemic damage, being released into the extracellular space after ischemic insult where it promotes neuroinflammation like neuropathy which represent a significant complication of diabetes and it is difficult to treat. Objective: is to investigate the changes of serum levels of High Mobility Group Box-1 Protein (HMGB-1) and its association with the incidence of developing neuropathic complications among diabetic patients.

Material & Methods: A 30 patients with diabetic peripheral neuropathy was included with another 30 patients with Diabetes Mellitus type 2 without complications and a 30 healthy subject as control group both sex-aged matched with the patients groups. This study included the measurement of serum high mobility group box -1 (HMGB-1) by ELISA technique in addition to HbA1c by turbidimetric immunoassay technology, Fasting blood sugar and finally Lipid profile (Total cholesterol, LDL, VLDL, HDL and Triglycerides) were measured by spectrophotometer. Results: Analyzing the current data of samples (n=90) showed that the comparison between serum level of HMGB-1 was significant. The mean of HMGB_1 for NPDM (689.08± 104.63) was significantly higher than both for Non-NPDM (354.97± 137.56), p < 0.001 and for control (312.91± 100.66), (p < 0.001). Also serum levels of (Total Cholesterol, TG, LDL, VLDL) of NPDM patients were significantly higher (p<0.001) than both Non-NPDM and control groups. We conclude that serum level of HMGB-1 can be used as predictor marker of neuropathy complication of type 2 diabetes.

Keywords: Serum, Box-1, Diabetic Peripheral Neuropathy

抽象的

背景：高迁移率组 B1 与中枢缺血性损伤有关，在缺血性损伤后被释放到细胞外空间。在那里促进神经炎症，如神经病变，这是糖尿病的重要并发症，难以治疗。目的：探讨糖尿病患者
血清高迁移率族Box-1蛋白（HMGB-1）水平的变化及其与发生神经病理性并发症的关系。材料与方法：30例糖尿病周围神经病变患者，另30例无并发症的2型糖尿病患者和30例健康受试者作为对照组，性别年龄与患者组相匹配。本研究包括通过ELISA技术测量血清高迁移率族框-1（HMGB-1）以及通过比浊免疫分析技术测量HbA1c，空腹血糖和最后的血脂谱（总胆固醇、低密度脂蛋白、极低密度脂蛋白、高密度脂蛋白和甘油三酯）用分光光度计测量。结果：分析样本（n=90）的当前数据表明，血清HMGB-1水平之间的比较是显著的。NPDM的HMGB_1平均值（689.08±104.63）显著高于非NPDM（354.97±137.56）、p<0.001和对照（312.91±100.66）(p<0.001)。NPDM患者的胆固醇、TG、LDL、VLDL）显著高于非NPDM组和对照组（p<0.001）。我们得出结论，血清HMGB-1水平可用作2型糖尿病神经病变并发症的预测指标。

关键词：血清，Box-1，糖尿病周围神经病变

1. Introduction

Diabetic Neuropathy are a group of clinical syndromes generated by injury to the peripheral and autonomic nerve systems that are by far the most common among diabetes complications. This study focused on diabetic peripheral neuropathy (DPN) as “a symmetrical, length dependent sensorimotor polyneuropathy connected to metabolic and microvessel changes as a result of chronic hyperglycemia exposure. This study will focus on the most prevalent type of diabetic neuropathy, distal symmetric polyneuropathy, and will be referred to as diabetic neuropathy throughout. Distal symmetric polyneuropathy demonstrates with a ‘stocking and glove’ distribution, the hands and lower limbs are the most frequently affected areas. Without appropriate intervention, one-third of the 97 billion people predicted to live in 2050 would have diabetes, and half of those will have neuropathy (1). Diabetes duration and hemoglobin A1c (HbA1c) levels are key predictors of diabetic neuropathy, as are other metabolic factors associated to the condition, mainly in T2DM, such as hypertension, Obesity, smoking, alcohol abuse, increased height and older age 30 is common in patients with neuropathy (2). Diabetic Peripheral Neuropathy complications are a major cause of morbidity and mortality in the diabetic population; accordingly, it is critical that DPN be recognized early in order to enhance glycemic control and improve patient quality of life. High mobility group B1 (HMGB1) is a DNA-binding protein that belongs to the High mobility group (HMGB1) superfamily, a group of ubiquitous non-histone nuclear proteins that regulates gene expression (3). It was discovered by Goodwin and Johns in 1973 and is characterized by high mobility in polyacrylamide gel electrophoresis (4). High mobility group (HMGB1) is a 30 kDA nuclear protein contains 215 amino acids having two N-terminal DNA-binding domains, called (BOX A and BOX B), also an acidic C-terminal tail. BOX B is, generally, in control of the pro-inflammatory effect stimulating the release of cytokines (5). High mobility group B1 has both a structural role and a role in DNA transcription,
replication and repair; it also contributes to nuclear proteins assembly. In the cytoplasm, it works as a signaling regulator and, in the extracellular milieu, it is involved in inflammatory cascade, acting as an “alarmin” and as a pro-inflammatory cytokine. In case of cellular damage or cellular death (HMGB1) is translocate outside the cell besides it was also obviously revealed that it can be actively secreted by stimulated immune cells such as monocytes, macrophages, mature dendritic (MD) cells, natural killer (NK) cells and endothelial cells as a result of different stimuli, for instance exposure to lipopolysaccharide (LPS), TNF-α, or IL-1β, IFN-γ and tissue injury. Moreover, it has been confirmed that oxidative stress stimuli the release of HMGB1. Oddly, the translocation from nucleus to cytoplasm, is assisted by JAK/STAT1 pathway, similarly translocation from cytoplasm to extracellular milieu can be determined by a mechanism facilitated by inflammasome activation during pyroptosis, a form of pro-inflammatory programmed cell death (6). Hyperglycemia stimulates the release of HMGB1 in diabetic patients, either directly or indirectly, through the formation of reactive oxygen species (ROS) and advanced glycation end products (AGEs) (7). The interaction of extracellular HMGB1 and its receptors (RAGE and Toll Like Receptors) this positive feedback loop contributes to the release of additional pro-inflammatory cytokines. This study aim to investigate the changes of serum levels of High Mobility Group Box-1 Protein (HMGB-1) and it is association with the incidence of developing neuropathic complications among diabetic patients.

2. Materials and Methods

2.1. Participants

This case-control study was conducted at Chemistry and Biochemistry Department/College of Medicine in collaboration with National Diabetes Center (NDC)/ Mustansiriyah University from October 2020 to April 2021 all patients and control were collected from National Center for Diabetes Research and Treatment Baghdad/Iraq. Ninety (90) samples included in this study and divided into three groups, two of which included type 2 diabetes patients, and they were divided, according to the bases of clinical examination and an Electromyography Nerve Test (EMG).

Exclusion criteria included: Patients with type1 diabetes, gestational diabetes, nephropathy, cardio vascular disease, chronic liver disease, patients with fever and those with acute infection on the day of sampling in addition to alcoholics and smokers. Inclusion criteria included fasting patients with Type 2 diabetes aged above 30. Healthy controls also underwent the same diagnostic and screening procedures as patients. Healthy controls could not have any history of ankle sprain within the past three years prior to enrollment.

2.2. Neurological examination

A medical evaluation involving review of medical history and pain symptoms and a detailed neurological examination were performed by trained study clinicians to confirm the presence of neuropathic pain.

We are following the (Toronto Clinical Neuropathy Scoring System) this is a quantitative scoring system for evaluating the severity of peripheral neuropathy primarily for the feet. Most of the testing is done on the toes or near them. Light touch testing is done with a 10 monofilament on the dorsum of the large toe done by a project-affiliated physician. A 128-
Hz tuning fork will be used to assess peripheral neuropathy, usually by comparing how long the patient detects vibration in comparison with the examiner. It determines whether vibration sense is normal, impaired, or absent. Finally an Electromyography Nerve Test (EMG) was done to patients with diabetic peripheral neuropathy; Electromyography (EMG) is a form of electro diagnostic testing that is used to study nerve and muscle function. It is commonly performed by a physiatrist or neurologist with special training for this procedure.

Samples
Six milliliters of blood taken from fasting patients and control groups each blood sample was divided into two parts; the first one: Two ml of blood transferred into EDTA containing tubes, for measurements of HbA1c. Second part: Four ml transferred into gel tubes, allow clotting for 30 minutes, after that the serum was separated by centrifugation at (3000 rpm) for (10 minutes) and divided into Eppendorf tubes and stored at (–20 °C) until the time of measurements of the studied biochemical parameters.

Laboratory Examination
High mobility Group B1 (HMGB-1) was measured by using a sandwich enzyme immunoassay kit for in vitro quantitative measurement of HMGB-1 in human serum manufactured by (mybiosource USA). Furthermore investigation were performed including first line laboratory exams performed which include the determination of HbA1c, it had based on turbidimetric immunoassay (TINIA) for hemolyzed whole blood, by automated system using kit manufactured by (dimeditec Germany). In addition, Fasting Blood Glucose (FBG) was measured by enzymatic colorimetric assay for the quantitative in-vitro diagnostic measurements, using kit supplied by (Biolabo, France). Finally lipid profile (Total Cholesterol, LDL, VLDL, TG, and HDL) was measured by spectrophotometry method using a kit manufactured by Human, Germany.

Statistical analysis: analysis of data was carried out by using the available Statistical Package for Social Sciences IBM SPSS Statistics 26.0 software (IBM SPSS Inc., Chicago, IL) and MedCalc@ version 19.5 were used for statistical analysis. The quantitative variables (measured parameters) were expressed as means ± standard deviation (SD), Minimum and maximum values, Medians and standard error of mean. An analysis of variance (ANOVA) was conducted to determine whether there were significant differences in different variables among the groups after Levene's test was conducted to determine whether the model residuals have similar variances between the groups of the independent variables. Then Post hoc. Tukey test were calculated between each pair of measurements to further examine the differences among the variables for all significant effects based on an alpha of 0.05. Chi square test was used to test the differences among categorical variables. (Student t test) was used to compare between means (P value ≤ 0.05 has been deemed to be statistically significant). Relationship between the variables was measured by Pearson linear correlation.

Results
This study include 90 participants both sex and aged matched the age range is from 30 to 77 years with mean age of neuropathy diabetic patients (NPDM) (57.87± 11.07 ) years and (59.80± 12.99) years for the non-neuropathy
diabetic patients (Non-NPDM). This study found that the results of the comparison of the BMI between the groups were significant; mean for the NPDM (31.55±4.91) kg/m2. On the other hand, the mean for the second group (Non-NPDM) was (31.57±5.49) kg/m2 while the mean for the control group was (28.92± 3.68.) kg/m2. This study found that the results of the comparison of the DM Duration were significant, the NPDM group mean was (15.80± 7.58.) years It is significantly higher than the mean of Non-NPDM results (5.20± 5.17.) years all are explained in Table 1-1.

Table 1-1 Summary Statistics for Age, BMI and diabetic duration by the studied groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean±SD</th>
<th>Min</th>
<th>Max</th>
<th>Median</th>
<th>P value ANOVA test</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>30</td>
<td>57.8±11.0</td>
<td>2.0</td>
<td>2</td>
<td>3.0</td>
<td>60</td>
<td>0.5</td>
</tr>
<tr>
<td>NPDM M</td>
<td></td>
<td>2.7±12.0</td>
<td>3</td>
<td>7.0</td>
<td>5.0</td>
<td>61.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Non-NPDM M</td>
<td></td>
<td>3.2±13.0</td>
<td>3</td>
<td>7.0</td>
<td>5.0</td>
<td>57.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>56.2±13.5</td>
<td>2</td>
<td>4</td>
<td>3.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>BMI (kg)</td>
<td>30</td>
<td>31.5±4.91</td>
<td>0.9</td>
<td>2.0</td>
<td>4.0</td>
<td>32.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Analyzing the current data of patients (n=90) showed for FBS analysis of variance were significant. The mean of FBS for NPDM (276.63± 67.72) mg/dl was significantly higher than for both Non-NPDM (195.00± 55.99) mg/dl, (p < 0.001) and control (91.37± 10.95) mg/dl, (p < 0.001). Additionally, the mean of FBS for Non-NPDM (195.00± 55.99) mg/dl was significantly higher than for control (91.37± 10.95) mg/dl, (p < 0.001).

Secondly, the analysis of variance for HbA1C was significant. Comparisons showed the mean of HbA1C for NPDM (8.84± 2.63) % was significantly higher than for control (6.06± 0.33) %, p < 0.001. The mean of HbA1C for Non-NPDM (8.80± 0.84) % was significantly higher than for control (6.06± 0.33) %, p < 0.001. All are explained in table 1-2.
Table 1-2 Descriptive Statistics for main markers in the neuropathic, non-neuropathic and control groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>$N = 30$</th>
<th>$Mean \pm SD$</th>
<th>$Min.$</th>
<th>$Max.$</th>
<th>$Median$</th>
<th>$P\ value$</th>
<th>Adjusted R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB Smg/dl</td>
<td>N P D M</td>
<td>276.63±67.24</td>
<td>2.18</td>
<td>9.45</td>
<td>3.76</td>
<td>26.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Non - NPDM</td>
<td>195.00±55.97</td>
<td>1.10</td>
<td>10.01</td>
<td>29.67</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>91.37±10.95</td>
<td>0.03</td>
<td>74.00</td>
<td>11.90</td>
<td>0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1C%</td>
<td>N P D M</td>
<td>8.84±2.63</td>
<td>0.04</td>
<td>0.09</td>
<td>12.02</td>
<td>2.2</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Non - NPDM</td>
<td>8.80±0.84</td>
<td>0.17</td>
<td>10.01</td>
<td>8.7</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>6.06±0.33</td>
<td>5.40</td>
<td>6.50</td>
<td>6.10</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>TG mg/dl</td>
<td>N P D M</td>
<td>204.03±7.30</td>
<td>3.03</td>
<td>10.6</td>
<td>17.59</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non - NPDM</td>
<td>143.20±87.68</td>
<td>6.01</td>
<td>37.5</td>
<td>11.4</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>106.80±8.41</td>
<td>5.28</td>
<td>54.14</td>
<td>10.7</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>VL DL mg/dl</td>
<td>N P D M</td>
<td>54.10±23.72</td>
<td>4.03</td>
<td>12.83</td>
<td>59.59</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non - NPDM</td>
<td>32.80±22.12</td>
<td>4.04</td>
<td>11.75</td>
<td>24.24</td>
<td>&lt;0.035</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>20.67±5.47</td>
<td>1.03</td>
<td>10.28</td>
<td>31.02</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Cholesterol mg/dl</td>
<td>N P D M</td>
<td>244.3±21.29</td>
<td>3.89</td>
<td>19.39</td>
<td>29.04</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non - NPDM</td>
<td>167.77±34.12</td>
<td>6.23</td>
<td>97.97</td>
<td>16.95</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>
The analysis of variance for lipid profile demonstrated that serum levels of (Total Cholesterol, TG, LDL, and VLDL) of NPDM patients were significantly higher than both Non-NPDM and Control groups ($p < 0.001$). In contrary, the data analysis of serum levels of HDL of NPDM patients were insignificant ($p=0.88$).

Lastly, analyzing the current data of patients (n=90) showed that the comparison between HMGB_1 serum level results was significant. The mean of HMGB_1 for NPDM (689.08± 104.63) pg/ml was significantly higher than both for Non-NPDM (354.97± 137.56) pg/ml, $p < 0.001$ and for control (312.91± 100.66) pg/ml, $p < 0.001$.

![Figure 1-1 HMGB-1 levels in neuropathic, non-neuropathic and control groups](image1)

![Figure 1-2 ROC analysis for HMGB-1](image2)

Figure 1-1 HMGB-1 levels in neuropathic, non-neuropathic and control groups

From the rock analysis in this study it can be found that in order to distinguish between diabetic neuropathy in diabetic neuropathic patients group and non-neuropathic diabetic...
patients group and the control by using the ROC analysis to regulate the parameter depending on AUC that can be occupied and if this occupation is significant or not.

As in figure (1-2) the level of (High mobility group box -1) of diabetic neuropathy group greater than or equal to 488.68 gp/ml with 100% specificity and 100% sensitivity and the (AUC=1000, p=<0.0001) that mean HMGB1 in NPDM group is a perfect marker for predicting neuropathy in type2 diabetes patients.

**Discussion**

High mobility group B1 is involved in the pathogenesis of all diabetic complications according to numerous studies. As a result of the increased morbidity and mortality associated with diabetic complications, as well as the discovery of new molecular pathways behind DM pathogenesis Thus, more research must be focused on developing new predictive markers for NPDM and preventing its morbidity and mortality(1). The relationship between HMGB1 and DPN has only been studied in a few researches. Diabetic neuropathy is a serious condition that is difficult to treat. There are not many analgesic agents that work well on neuropathic pain without serious side effects. Many studies have found that inflammation might play a role in painful diabetic neuropathy. Data analysis of the current study indicated that the mean of HMGB-1 for Neuropathic Diabetes Militias patients (NPDM) was significantly higher than both for Non- Neuropathic Diabetes Militias (Non-NPDM) patients and for control. In addition to that, the data of the Non-NPDM patients were significantly higher than the data of the control group. This results were in agreement with Dasu and college (8) who's showed that circulating levels of HMGB1 were higher in type 2 diabetic patients than control, and the same phenomenon was observed by Škrha Jr, J. and college (9).

In diabetic condition, high glucose or AGEs (Advanced Glycation End-products) can induce HMGB-1 resection via oxidative stress. HMGB-1-RAGE interaction stimulates islet cell apoptosis in diabetes by inducing oxidative stress, having a major role in increasing insulin deficiency. Moreover, released HMGB-1 stimulates (c-Jun N-terminal kinase/ p38 mitogen-activated protein kinase) JNK/p38MAPK activation through binding to TLR4, contributes into insulin resistance via inhibitory phosphorylation of insulin receptor substrate. As a final point, HMGB-1-induced insulin deficiency and insulin resistance lead to the development of diabetes. (3).

Despite the fact that many mechanisms have been studied, not all participants responsible for these complications have been defined yet but, (HMGB1) is a non-histone nuclear protein that has been involved in numerous pathological processes, beginning from sepsis to ischemia (1). The passive release of HMGB1 from damaged cells or activated immune cells following infection, injury, and sterile inflammation also determines the guideline of inflammatory responses (10),( 11).

Studies have implicated that neuroinflammation and the progress of neuropathic conditions greatly depend on high mobility group box1 protein that plays a fundamental role in developing these conditions, HMGB1 is a proinflammatory cytokine that can be released from necrotic cells in passive form or in response to inflammatory signals as an active form. (12). Previous studies have shown that the release of IL-1α, IL-1β, and IL-6 in cultured human primary macrophages stimulated by HMGB1
high mobility group box1 might initiate downstream signaling via the receptor for advanced glycation end products (RAGE), and through toll-like receptors, TLR2 and TLR4. The data analysis demonstrated that patients with Diabetic Periphal Neuropathy presented a higher (Total Cholesterol, TG,LDL and VLDL) levels than both the non-neuropathic diabetic patients and the controls, more over the data results of Non- NPDM are higher than the control. The same phenomenon was observed by Cai, Z., Yang, Y., and Zhang, J. (14) also it support the hypothesis that serum lipid profile changes are among the biological characteristics of Diabetic Peripheral Neuropathy. On the other hand, The study results of serum levels of HDL indicated that there were insignificant differences between the groups ($p = 0.88$). There is a high incidence of dyslipidemia in T2DM patients, and dyslipidemia is linked to Diabetic Peripheral Neuropathy. Quite a lot of pathological modifications in neurons, glia and vascular cells, which can lead to nerve dysfunction and ultimately DPN caused by hyperglycaemia and dyslipidaemia, together with altered insulin signalling (14).

**Conclusion**: Serum level of HMGB-1 of the neuropathy patients was significantly higher than both non-neuropathy patients and control which indicate that this marker is perfect to predict Diabetic Peripheral Neuropathy. As well as lipid levels should be investigated as a standard laboratory marker for predicting Diabetic Peripheral Neuropathy risk as they will assist physicians in selecting appropriate therapies and therefore optimizing the use of available resources.


