Investigation of Erythrocyte membrane average roughness in type 2 diabetes patients and guinea pigs

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Abstract: Diabetes mellitus (DM) is non-communicable chronic hyperglycemia caused by metabolic disorder of the endocrine system. Type 2 diabetes accounts more than 90% of diabetes. ROS act to damage the cell membrane proteins and lipid. Erythrocytes are the most common type of blood cells in vertebrates. The erythrocytes have longer life span than other cells, so they more expose to accumulation of glycation end products. Erythrocyte membrane average roughness using AMF, FPS, and HbA1C determined. Non-significant value \(p>0.073\) when compared LT with ST group (21.63±4.15 and 22.53±4.91) while the both groups has significant \(p<0.0001\) increase in average roughness when compared with control. Significant increase in average roughness of erythrocyte membrane of guinea pigs in diabetes group (21.21±8.62) compared with control (9.23±4.27).

Introduction

Diabetes mellitus (DM) is non-communicable chronic hyperglycemia caused by metabolic disorder of the endocrine system that affect the metabolism of lipid, carbohydrate and proteins. (Navin S., and Feray G., 2020). It's caused by impaired insulin secretion, signaling and discontinues of insulin synthesis (ADA, 2019). Individual suffer diabetes expected to be 642 in 2040 (WHO, 2019). Diabetes classify into type1 also known insulin dependent, type2 also known insulin independent gestational, and specific type of diabetes Type 2 diabetes accounts more than 90% of diabetes (ADA, 2019). Aging, ethnicity, family history, low socioeconomic status, obesity, metabolic syndrome, and certain unhealthy lifestyle behaviors are risk factors that are implicated in the pathogenesis of type 2 diabetes (Zheng Y, \textit{et al}, 2018). Elevated and uncontrolled of hyperglycemia definitely lead to complication of diabetes such as cardiovascular disease, retinopathy, nephropathy, neuropathy and hypertension (Grieco, G., \textit{et al}, 2019). Complications occur during increase the level of ROS that caused by hyperglycemia in which ROS act to damage the cell membrane proteins and lipid in addition its change the metabolic pathway of cells and organs that lead to significant change in membrane proteins (Aleksandra C., \textit{et al}, 2020; Vadvalkar S., \textit{et al}, 2017). Erythrocytes are the most common type of blood cells in vertebrates, and carry oxygen throughout the body. These erythrocytes are a product of a differentiation process that initiate in the bone marrow. Erythrocytes are develop into mature erythrocyte with biconcave disk after degradation of nuclei and endoplasmic reticulum. Erythrocyte membrane content is similar to most of mamalian's membranes and it is composed by: 19.5% of water, 35.1% of lipids, 5.8% of carbohydrates, and 39.5% of proteins (Dina B, \textit{et al}, 2021; Eitan F., 2021; Monje V., and Klauda, J., 2016).

The erythrocyte membrane skeleton is a complexes of proteins formed by multiple structural proteins such as ankyrin, \(\alpha\) and \(\beta\), spectrin protein 4.1, band3 and actin. The membrane skeleton proteins interact each other, transmembrane proteins, and lipid bilayer to give RBC membrane strength and flexibility (Deborah M., \textit{et al} 2021; Martin, J. and Sawyer, A., 2019; He L., and George L., 2014). The erythrocytes have longer
life span than other cells, so they more expose to accumulation of glycation end products when presence in high glucose concentrations during diabetes. Elevated level of glucose lead to enzymatic and non-enzymatic glycation of plasma and extracellular membrane proteins due to defect in metabolism of glucose (Marcello A., et al, 2020; Georgiana R., and Silvana A., 2016). This defect change in metabolism protocol of RBCs that lead to increase the activity of ROS offset by decrease in antioxidant activity thereby damage of proteins. The damage cause further change in RBC membrane proteins lead to complication involved macro and micro-vascular disease (Shehan N., et al, 2019; Zangeneh M, et al, 2018).

Aim of study is to evaluate the erythrocyte membrane roughness in type 2 diabetes patient using atomic force microscopy (AFM)

Material and method
This study involve two part of sample first 127 individual participate in current study divided into three groups as apparently healthy as control n=60, more than 10years with diabetes (long term (LT)) n=36 and less than 10 years with diabetes (short term (ST)) n=31 as The whole blood sample of 67 diabetes patients collected in Al-yarmuk hospital and placed in EDTA tube. The second part consisting of 12 male guinea pigs with 36 weeks old with average weight 0.485±0.054 g provided by Iraqi national center for drug control and research. The animals were housed in poly vinyl cage with conditions 12 h/12 h light/dark, 24 °C, and free access to food and water for 2 weeks. Diabetes induced in 6 animals by daily injection of 3mg/kg of dexamethasone followed by orally 5 ml of 50% sugar solution for 5 weeks (group A). The remaining 6 guinea pigs injected with normal saline as control (group B). The fasting plasma sugar (FPS) determined in both part using RANDOX sugar kit, HbA1C. The HbA1C determined in human whole blood using fluoroimmuno assay (FIA) technique average roughness of erythrocyte membrane determined using AFM, in which 100 µl whole blood smeared on slid and dried at 30 °C for 15 minutes followed scratch the dried blood gently and collected as powder in eppendorf tube for AFM assay.

Results and discussion

1-Human sample: The FPS and HbA1C determined in two patients group and compared with control as shown in Table (1)

Table 1: mean±SD of FPS and HbA1C in long-term (LT) and Short term (ST) compared with control (t-test)(A), mean±SD of FPS and HbA1C in long-term (LT) and Short term (ST) diabetes groups (ANOVA study) (B)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Control</th>
<th>LT</th>
<th>ST</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPS</td>
<td>mg/dl</td>
<td>91.08±13.66</td>
<td>258.92±84.28</td>
<td>273.34±74.25</td>
<td>&lt; 0.000</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>HbA1C</td>
<td>%</td>
<td>5.39±1.08</td>
<td>11.30±1.84</td>
<td>11.60±1.75</td>
<td>&lt; 0.000</td>
<td>&lt; 0.000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>ST</th>
<th>LT</th>
<th>p -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPS</td>
<td>mg/dl</td>
<td>91.08±13.66</td>
<td>273.34±74.25</td>
<td>0.321</td>
</tr>
<tr>
<td>HbA1C</td>
<td>%</td>
<td>5.39±1.08</td>
<td>11.60±1.75</td>
<td>0.939</td>
</tr>
</tbody>
</table>
The results expected to be significant \( p>0.000 \) increase in FPS and HbA1C in both patient groups (LT and ST), while FPS and HbA1C show non-significant differences when compare LT with ST group.

The hyperglycemia may attribute to both \( \beta \)-cell dysfunction and/or insulin resistance. (Aurora M., \textit{et al}, 2021; Latha S., \textit{et al}, 2019; Ronald g., \textit{et al}, 2018; Pia V. \textit{et al}, 2016). The elevated level of HbA1C is caused by non-enzymatic glycation of hemoglobin due to hyperglycemia (Wenjia G., \textit{et al}, 2019; Kerry J., \textit{et al} 2016; Shiva R., 2018). It is produced by ketamine reaction between glucose and N-terminal of valine of both \( \beta \) chains of the hemoglobin molecule (Zhenzhou W., \textit{et al}, 2020; Junhaeng L., \textit{et al}, 2020). Glucocorticoids have been shown to inhibit a number of steps in insulin signaling, causing insulin resistance. Insulin resistance affect metabolism of, liver, muscle, adipose tissue and brain, as result, reduction in insulin sensitivity and insulin resistance by reduction transcription of insulin receptor substrate1 (IRS-1), it's also decrease IRS-1 and 2 levels in adipose tissue while its lowering phosphorylation of IR and IRS-1 in liver (Leili R., \textit{et al}, 2020; Alex R., \textit{et al}., 2014). Insulin resistance causing excess insulin secreted from the pancreas to maintain blood glucose but serum glucose remains elevated due to effect of glucocorticoid followed by depletion in \( \beta \)-cells mass (apoptosis) (Marlon E., 2020; Jun Y., \textit{et al}, 2018; Nicholas H., \textit{et al}, 2018).

2-Animal sample: FPS determined in guinea pigs and the results show significant \( p>0.000 \) decrease in diabetes group compared with control with mean ± SD 84.26±15.65 204.67±18.82 respectively. As shown in Figure (1) there is depletion in \( \beta \)-cell in B group compare with A due to inhibitory effect of dexamethasone on insulin signaling that causing insulin resistance followed by decrease in synthesis of insulin receptor substrate, so more insulin will secret without response to blood sugar, chronic elevated insulin level without lowering of blood sugar lead to reduction in \( \beta \)-cell mass. (Carine B., \textit{et al}, 2021; Junhaeng L., \textit{et al}, 2020).

\[ \text{Figure 1): H and E micrograph of pancreas section 10x, of Negative control (A), Positive control (B).} \]
Determination of human erythrocyte membrane average roughness: The average roughness of erythrocyte's membrane determined using atomic force microscopy (AFM) and the results indicate non-significant value $p>0.259$ when compared LT to ST group with mean±SD 21.63±4.15 and 22.53±4.91 respectively while both groups show significant $p<0.0001$ increase in average roughness when compared with control group (11.03±5.02). Figure (2) show the AFM micrograph of erythrocytes of control, LT, and ST groups. This results were disagree with (Sunita S., et al, 2019 and Theresa P., et al, 2015 ) who found decrease of RBC roughness in diabetes group, while the results were agree with (Visser. J et al, 2017; Pretorius E., et al, 2015) they found increase average roughness of erythrocyte membrane in diabetes group. Several studies mention the change of erythrocyte membrane properties in diabetes and other disease such as malaria, sickle cell anemia, diabetes, myocardial infarction, and paroxysmal nocturnal hemoglobinuria (Adrita B., et al, 2020; visser J., et al, 2017).

![Figure 2: AFM micrograph of erythrocyte in control (A), LT (B), and ST (C) groups. A1, B1, and C1: 2 dimension micrograph 0.0 - 3000 nm. A2, B2, and C2: 3 dimension micrograph 0.0 - 2550 nm. A3, B3, and C3: diagram of corresponding 3D micrograph of erythrocyte surface roughness.](image-url)
Determination of guinea pigs erythrocyte membrane average roughness: The average roughness of erythrocyte membrane determined in A and B group Figure (3). The results indicate significant \( p>0.000 \) increase in diabetes group (group B) compare to Control (A) with mean and SD 21.21±8.62 and 9.23±4.27.

Figure 3: AFM micrograph of erythrocyte in control (A), diabetes (B) groups. A1 and B1: 2 dimension micrograph 0.0 - 3000 nm. A2 and B2. 3 dimension micrograph 0.0 - 2550 nm. A3 and B3. Corresponding 3D micrograph of erythrocyte surface roughness.

This results is agree with (Mohamad S., et al, 2018; Theresia P., et al, 2015). Adrita and her coworker found that protein contents of erythrocyte membrane is increased in diabetes, they also found increase of phospholipid contents in diabetes group about 40% more than control(Adrita et al, 2019). Under physiological conditions, erythrocytes able to defend against oxidative molecules , by converting of oxidant or toxic molecules to non-toxic in forms that can be used by cell or excreted. However, under diabetes condition abnormal response of cell to oxidative stress can produce of peroxides and free radicals, leading to damages in erythrocyte skeleton, membrane fluidity, and cellular signaling (Cristiana C., and Francesco M., 2017). Elkrief and coworkers found that high level of advance glycation end product (AGE) in diabetic erythrocytes affect protein integrity involved in membrane structure (Elkrief et al., 2016). Hatanaka and colleagues prove that elevated level of erythrocyte protein glycation resulting in higher fragility of cells due to affecting skeleton membrane proteins such as Actin, ankyrin, band 3 and 4.1, and spectrin. (Banerjee, A., et al, 2020). The disorders mentioned above that occur in diabetes, can be deduced that why the surface of the roughness of the red blood cells has increased.

Conclusion

1- Increasing the erythrocyte membrane average roughness in diabetes patient.
2- HbA1C independent on duration of diabetes (ages) as results in current study that show there was non-significant differences found between group with long and short term of diabetes and this results not contradic with fact that long term elevated
HbA1C leading to complications, but even with complication the HbA1C can be reduced by treatment and diet.

3- Low cost and un-consuming time of converting whole blood to powder under 30 ° C give acceptable results when determined with AFM.

Acknowledgement

Arthurs very thankful to many important papers from talented people who made and found significant facts to erythrocyte research. They also gratefully thanks of department of chemistry - collage of science - Al-Mustanseryah University, Iraqi national center for drug control and research, and Baghdad University who help to facilitate this study.

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