ASSESSMENT OF TOTAL RNA-BINDING PROTEINS LEVELS AS BIOCHEMICAL MARKER IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background: Type 2 diabetes, which used to be called adult-onset diabetes, can affect people at any age, even children. Recent advancements in RNA-based technologies indicate that RNA-regulatory networks controlled by RNA binding proteins (RBPs) are modulated in diabetes and contribute to systemic manifestations of diabetes.

Subjects and Methods: The diabetic group who subjected to this study were (45) of type 2 diabetic patients ages of them were range between (30-55) years, which were divided into two groups according to the gender (22) male and (23) female, the control group also, includes 45 persons apparently healthy, they were free from symptom and signs of any diseases and ages of this group was ranged between (30-55) years, which were also, divided according to the gender (23) female and (22) male. Determination of total RNA binding protein concentration is done by Sandwich-ELISA kits by Sunlong (China) Company.

Results: The results revealed that there was a significant differences in the total concentration of RNABPs between patients and control groups (p-value <0.05), the results also revealed that there was no significant difference in RNABPs concentration between female T2DM and its control (p >0.05), while the results showed that there was a significant difference between T2DM male and its control group (p< 0.05).

Conclusion: In our study we measure the total concentration of RBP it's important for future study to measure each type of RBP with a separate form to find the role of each one in T2DM occurrence and complications.

Keywords: T2DM, RNABPs, RNA Binding proteins, ELISA

I. INTRODUCTION:

The term diabetes describes a group of metabolic disorders characterized and identified by the presence of hyperglycemia in the absence of treatment. The heterogeneous aetio-pathology includes defects in insulin secretion, insulin action, or both, and disturbances of carbohydrate, fat and protein metabolism. Diabetes may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. Genital yeast infections frequently occur (1). Type 2 diabetes, which used to be called adult-onset diabetes, can affect people at any age, even children. However, type 2 diabetes develops most often in middle-aged and older people. Type 2 diabetes usually begins with insulin resistance condition that occurs when fat, muscle, and liver cells do not use insulin to carry glucose into the body’s cells to use for energy (2). RNA binding proteins and their networks RNA binding proteins (RBPs) are key components in RNA metabolism. They regulate all aspects of RNA biogenesis from RNA maturation, surveillance, nucleocytoplasmic transport to subcellular localization, translation and RNA degradation. RBPs form dynamic interactions with coding, untranslated and non-protein-coding RNAs in functional units called ribonucleoprotein (RNP) complexes (3). This enables the RBPs within RNP complexes to remain stably bound to the RNA throughout its journey from synthesis to degradation or to associate with the RNAs in a temporal and spatial manner (4). A well-known example of the dynamic interaction of RBPs is illustrated by the exon junction complex (EJC): an RNP complex containing at least ten proteins. The EJC is located 24 nucleotides upstream of exon–exon junctions and remains bound until the RNA is translated in the cytoplasm (5). Studies on RNP assembly in different species support the view that mRNAs that encode proteins with similar functions can be bound by the same RBP (6). The coordination of posttranscriptional events by RBPs in this manner forms the basis of the RNA operon hypothesis and has enabled a better understanding of RNP components and their target mRNA networks (7). The functions of RNA binding proteins in eukaryotes seen in...
(Figure 1). (a) The mRNA pathway. The nascent pre-mRNAs transcripts are generated transcriptionally by RNA polymerase II (Pol II). They are processed into mature mRNAs by a series of steps including the removal of introns during pre-mRNA splicing and the addition of a 30 polyadenylation tail. During the course of these events, RNA-binding proteins (RBPs) bind in a dynamic manner to the pre-mRNAs and mRNAs. The splice junctions are bound by the exon junction complex (EJC), and this regulates the cytoplasmic fate of the mRNAs (5). The mRNAs bound with RBPs and other factors including associated proteins and RNAs are assembled into mature mRNPs (6). The EJC is involved in preventing subsequent rounds of protein translation and the generation of deleterious proteins (7). A functional mRNA has its 7-methylguanosine cap structure bound by eIF4E and its polyadenylation tail bound by the poly(A) binding protein (PABP). This is followed by mRNA circularization and protein translation (8, 9). (b) The miRNA pathway. The biogenesis of miRNAs starts with nascent primary microRNAs (pri-miRNA) transcripts produced by RNA Pol II and processed by the RNase III enzyme Drosha complex into precursor miRNAs (pre-miRNAs). The pre-miRNA is exported by Exportin 5 and in the cytoplasm, pre-miRNAs are further processed by RNase III enzyme/dsRNA binding protein Dicer into mature miRNAs. One strand of the miRNAs is incorporated into the effector complex RNA-induced silencing complex (RISC) forming a miRNA that recognizes specific targets through imperfect base-pairing and induces posttranscriptional gene silencing (10). RBPs are dysregulated under diabetic conditions and their contribution to disease pathogenesis. New therapies that target RBPs for treatment of human diseases (11).

II. SUBJECT AND METHODS:

T2DM Patients: This study was performed at the laboratory of college of medicine-Babylon university. The collection of samples was conducted during the period 1st of November 2020 till 30th of March 2021. All patients of were diagnosed by physicians and samples were collected from Merjan Teaching Hospital in Hilla city. The diabetic group who subjected to this study were (45) of type 2 diabetic patients ages of them were range between (30-55) years, which were divided into two groups according to the gender; 23 female of type 2 diabetic patients and 22 male of type 2 diabetic patients.

Control: The control group includes 45 persons apparently healthy, they were free from symptom and signs of any diseases and ages of this group was ranged between (30-55) years, which were also, divided into two according to the gender into 22 male and 23 females.

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients’ verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee (College of Medicine/University of Babylon).

Collection of the blood samples

Five ml of venous blood were withdrawn from patients and control by venipuncture pushed slowly into gel tubes. Blood was allowed to clot at room temperature for 30 min and at 2000 $\times g$ for 5 min, then the serum was divided into small Eppendorf tube and kept at (+20° C) to be used later for biochemical estimation of total RNA binding protein concentrations by ELISA technique.

Determination of Total RNA binding protein concentration

Sandwich-ELISA kits by Sunlong (China) Company was used in this study as a method. Known concentrations of Human total RNA binding protein Standard and its corresponding reading OD are plotted on the log scale (x-axis) and the log scale (y-axis), respectively, Figure 2. The concentration of Human total RNA binding protein in the sample is determined by plotting the sample’s OD on the Y-axis. The original concentration is calculated by multiplying the dilution factor.
Figure (2): Standard curve for determination of Total RNA binding protein concentration

**Statistical Analysis:**
All the numerical data were expressed as mean ± SD. Descriptive statistics and graphs were carried out by using SPSS 19 (SPSS, Chicago, Ill., USA). Also, parameters mean ± SD were compared by using Student t test.

**Results:**
The results revealed that there was a significant difference in the concentration of RNABPs between patients and control groups (p-value < 0.05). The means, standard deviation, and statistical parameters are listed in the table (1). The results also revealed that there was no significant difference in RNABPs concentration between female T2DM and its control (p > 0.05), while the results showed that there was a significant difference between T2DM male and its control group (p < 0.05). Correlation between age, BMI, and RNABPs revealed significant weak negative correlation between age and RNABPs concentration p value = 0.016, r = -0.26 as seen in figure (4). Also, correlation was non-significant between BMI and RNABPs p value = 0.72, r = -0.04 figure (5).

| Group | N  | Mean±SD pg/ml | P-value
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>RNABPs</td>
<td>T2DM</td>
<td>45</td>
<td>1908.4±317.4</td>
</tr>
<tr>
<td>Control</td>
<td>45</td>
<td>2253.2±927.4</td>
<td></td>
</tr>
</tbody>
</table>

Table (1): RNABPs Concentration for Patients and Control Groups.

Figure (3): Comparison in Mean of RNABPs Between T2DM Patients and Control
Table (2): RNABPs Concentration for Female T2DM and Control Groups.

<table>
<thead>
<tr>
<th>Female Group</th>
<th>N</th>
<th>Mean±SDpg/ml</th>
<th>P-value*</th>
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<tbody>
<tr>
<td>RNABPs</td>
<td>T2DM</td>
<td>23</td>
<td>1936.6±352.6</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23</td>
<td>2038.8±434.0</td>
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Table (3): RNABPs Concentration for Male T2DM and Control Groups.

<table>
<thead>
<tr>
<th>Male RNABPs</th>
<th>N</th>
<th>Mean±SDpg/ml</th>
<th>P-value*</th>
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<tbody>
<tr>
<td>T2DM</td>
<td>22</td>
<td>1877.6±279.3</td>
<td>0.035</td>
</tr>
<tr>
<td>Control</td>
<td>22</td>
<td>2255.6±738.8</td>
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Figure (4): Correlation between age and RNABPs concentration.

Figure (5): Correlation between BMI and RNABPs concentration.
III. DISCUSSION:

In this study, the result revealed the presence of significant difference between all T2DM patients and control, and between male T2DM and control, the RNABPs concentration in T2DM patients is lower than control, except in female T2DM and control, there was no significant difference between them. Recent studies demonstrate that RBPs play key roles in the development of diabetes and its systemic manifestations. The technological advancements in identification of global RNA targets of RBPs in a wide variety of tissues will help determine the major RNA regulatory programs disrupted under diabetic conditions. In summary, posttranscriptional regulation by RBPs is emerging as a key mechanism in the development and pathogenesis of diabetes and has the potential to provide new therapeutic options for diabetic patients (12). In order to fully understand the mechanisms shaping gene expression patterns in β-cells, particularly during disease relevant stress conditions, the repertoire of RBPs that impact β-cell differentiation, viability, and function will need to be elucidated. Given the central role of proinsulin production, processing, and secretion, much of our early understanding of RBP function in β-cells has come from the study of proinsulin biosynthesis. For example, the RBP polyuridine tract-binding protein (PTB) enhances the stability of proinsulin mRNA via binding to its 39 UTR (13). Beyond the regulation of proinsulin biosynthesis, RBPs also establish secretory function of β-cells via regulation of genes involved in various components of the insulin secretion pathway (14).

IV. CONCLUSION:

In our study, we measure the total concentration of RBP. It's important for future study to measure each type of RBP with a separate form to find the role of each one in T2DM occurrence and complications.

Conflict of interest

No potential conflict of interest relevant to this manuscript was reported.

REFERENCES: