

Original Research Article

Evaluation The Serum Total Protein in Patients with Diabetes Mellitus (Type I and Type II) and Study Genetic Level of Glutathione-S-Transferase μ 1

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Abstract

Diabetes mellitus is a group of metabolic diseases due to defect in insulin secretion or action or both. Hyperglycemia in diabetes creates free radicals. These free radicals produce oxidative stress and thus debilitate the endogenous antioxidant defense system. If the amount of insulin available is insufficient, cells respond badly to the effects of insulin or if the insulin itself is abnormal. The net effect is persistently elevating levels of blood glucose, low protein synthesis.

The current study evaluates the biochemical changes in diabetes mellitus patients in using different medications and investigate the glutathione S-transferase M1 gene deletion in different treatments.

The present study was conducted on (75) diabetic patients, (25) of them were treated with insulin, (25) were using insulin and metformin and the last (25) were on metformin and glibinclidide. The study also included (25) apparently healthy subjects were taken as control group. The blood samples were collected from Merjan Teaching Hospital from November 2013 to April 2014. The total serum protein was measured by colorimetric method described by Gornall.

LSD test showed that there was significant difference between insulin & metformin group and metformin & glibinclidide in total serum protein ($p \leq 0.05$). Metformin & glibinclidide group was less mean difference with the control group. In the gene level of the study, genotyping of glutathione S-transferase μ 1 gene by PCR were defined as GSTM1 and GSTM0 or deletion association to the present and absences of the guanine nucleotide in the gene sequence. There was statistically significant difference in the genotyping distribution and the frequency of GSTM0 among study groups were 44% for insulin and metformin group, 68% for metformin and glibinclidide, 44% insulin and 28% for control healthy group.

there was significant decrease in serum total protein in diabetic patients. Genetic polymorphism of glutathione S-transferase μ 1 gene may be considered as risk factor for both types of diabetes mellitus.

Key words: Diabetes mellitus, GST, GSTM1, Serum total protein.

الخلاصة

داء السكري هو مجموعة من الأمراض الاستقلابية بسبب خلل في إفراز الأنسولين أو الإجراءات أو كليهما. ارتفاع السكر في الدم في مرض السكري يخلق الجذور الحرة. هذه الجذور الحرة تنتج الأكسدة بالتالي إضعاف نظام الدفاع المضاد للأكسدة الذاتية. إذا كانت كمية الأنسولين المتاحة غير كافية، بشدة الخلايا استجابة الخلايا يكون سبباً لآثار الأنسولين أو إذا كان الأنسولين في حد ذاته هو غير طبيعي. الأثر الصافي هو ارتفاع مستويات الجلوكوز في الدم، تخليق البروتين بصورة منخفضة.

إن الدراسة الحالية تقييم التغيرات بالكمياء الحيوية في المرضى الذين يعانون مرض السكري في استخدام الأدوية المختلفة، وبالتحقيق في الحذف
الحاصل بالجلوتاثيون اس ترانسفيريز بالعلاجات المختلفة.

قد أجريت هذه الدراسة ب (٧٥) من مرضى السكري، (٢٥) من هم يعالجون بالأنسولين، (٢٥) كانوا يستخدمون الأنسولين والميتفورمين وآخرون (٢٥) كانوا على ميتفورمين وجلبينكليمايد. شملت الدراسة (٢٥) على ما يبدو كانوا أصحاء تؤخذ كمجموعة ضابطة. عينات الدم تم جمعها من مستشفى مرجان التعليمي من نوفمبر ٢٠١٣ إلى أبريل ٢٠١٤. البروتين الكلي في الدم كان يقاس بالطريقة اللونية التي وصفها Gornall. أظهر اختبار LSD الفارق الواضح بين مجموعتي الأنسولين & الميتفورمين و الجلبينكليمايد في مستوى البروتين الكلي في مصل الدم (ع ≥ 0.05). مجموعة الميتفورمين والجلبينكليمايد كانت فرق المتوسط أقل مع المجموعة الضابطة. في المستوى الجيني للدراسة، التتميط الجيني من الجلوتاثيون S-ترانسفيرازمو بواسطة PCR تم تعريفها GSTM1 الحذف GSTM0 متصاحبه بوجود او غياب النوكليوتيداتجوانين في التسلسل الجيني. حيث كان هناك إحصائي الفرق التتميط الجيني في توزيع وكانت وتيرة GSTM0 بين مجموعات الدراسة ٤٤٪ للأنسولين ومجموعة الميتفورمين، ٦٨٪ للميتفورمين والجلبينكليمايد، و ٤٤٪ أنسولين و ٢٨٪ للمجموعة المراقبة الصحية. هناك انخفاض كبير في البروتين الكلي في الدم في مرضى السكري. ويمكن اعتبار تعدد الأشكال الجيني للجلوتاثيون S-ترانسفيرازمو ١ gene كعامل خطر لكلا النوعين من مرض السكري.

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1].

If the amount of insulin available is insufficient, cells response badly to the effects of insulin (insulin insensitivity or insulin resistance) or if the insulin itself is abnormal, then glucose will not be absorbed by the body cells properly that need it, and it will not be stored in the liver and muscles normally. The net effect is persistently elevating levels of blood glucose, low protein synthesis, and other metabolic derangements, such as acidosis [2].

Glutathione S-transferase (GST) is group of enzymes that assumes a vital part in detoxification of xenobiotics. GST catalyzes attachment of the thiol of glutathione to electrophiles[3].

Cytosolic GSTs are classified into 13 classes according to their structures: alpha, beta, delta, epsilon, zeta, theta, mu, nu, pi, sigma, tau, phi, and omega [4].

A physical map has been developed of the human class Mu GST genes on chromosome 1p13.3. The GST genes in this

group are dispersed around 20 kilo base pairs (kb) separator, and orchestrated as 5*-GSTM4-GSTM2-GSTM1-GSTM5-3*.

This map has been utilized to confine the end purposes of the polymorphic GSTM1 cancellation [5].

Aim of this study was to evaluate the total serum protein changes in diabetes mellitus patients in using different medications and investigate the glutathione S-transferase M1 gene deletion in different treatments.

Material and Methods

The diabetic grapes who subjected in this study were divided into (3) grapes each group had (25) patients: First group had treated with insulin, their age ranging from (14-77) years, second group had treated with insulin accompanied with metformin, their ages ranging from (18-64) years, third group would treat with combination of oral hypoglycemic drugs metformin and glibinclidide. All the patients had suffered from DM for about 5-15 years, they should be not smokers or pregnant, they had no other chronic disease like hypertension and they had taken medications of DM regularly.

1. Serum Total Protein

The total serum protein was measured by colorimetric method described by Gornall. The peptide bonds of proteins react with Cu^{+2} in alkaline solution to form a coloured complex which absorbance, proportional to the concentration of total protein in the

specimen, is measured at 550 nm. The biuret reagent contains sodium potassium tartrate to complex cupric ions and maintains their solubility in alkaline solution.

2. Gene Level

In this experiment, Primers were designed based on the cDNA sequence for human GST μ . The primers hybridize to the 5' region of exon 4 (5'-CTGCCCTACTTGATTGATGGG-3') and the 3' region of exon 5 (5'-CTGGATTGTAGCAGATCATGC-3') of GST μ_1 [6].

Poly chain reactions had been occurred by mixing 12.5 μ l of master mix GoTaq® Green Master Mix (Promega-Green Master Mix), 2 μ l of primer mix, 4 μ l of DNA extraction (200 ng) and 6.5 μ l of deionized distilled H₂O. Net volume is 25 μ l. Primer mix consisted of 10 μ l of first primer and 10 μ l of second primer and complete

volume to 100 μ l with deionized distilled H₂O. Reactions were heated for 2 min at 94°C, 1 min at 55°C and 1.5 min at 72°C for 35 cycles in a Thermal Cycler. PCR products 273bp were electrophoresed on an 8% acryl amide gel.

Results and Discussions

1. Serum Total Protein

There was a highly significant mean difference of serum total protein (p value \leq 0.01) when compare three treated groups with control group using ANOVA test (Table 1).

The results showed significant means difference within treated diabetic groups, by using LSD test which showed that there is significant differences between Metformin & Glibinclidide and other treated groups; p = 0.044 (\leq 0.05).

Table 1 : Mean difference of total protein by study groups

Variable	Groups	Mean \pm SD	P value
Total Protein	Control	52.67 \pm 1.75 g/L	<0.01 *
	Insulin&Metformin	39.45 \pm 6.58g/L*	
	in&Glibinclidide	44.13 \pm 6.78g/L* ^s	
	Insulin	41.87 \pm 9.40g/L*	

*p value \leq 0.05 is significant in compare with control

^sp value \leq 0.05 is significant within treated groups

The diabetic patients suffer from significant decrease in serum TP as a result of chronically elevated blood glucose leads to the formation of advanced glycosylated products with subsequent hyperfiltration (a potential increase of 5%-10% in GFR) and glomerular hypertrophy [7] and that is agree with Evans and Capell who demonstrated that pathophysiologic components of diabetic nephropathy are not entirely seen yet incorporate glycosylation

of circulating and intrarenal proteins, hypertension, and anomalous intrarenal hemodynamics[8].

In the glomerulus of diabetes, protein passes across the endothelial cells of the glomerular capillaries, across the basement membrane and through the slit pores of the glomerular epithelial cells (podocytes) into Bowman's space [9].

The metformin & glibinclidide group was the lowest mean difference from

control group as a result of many populations NIDDM develops much later protein urea and less decrease in serum total protein and the risk of death from non-renal causes is much higher than in patients with IDDM of similar duration [10].

Albumin excretion typically increased in a continuous manner over several years, the average increase in albumin excretion rate and decrease in TP range from 10 to 30% per year until over nephropathy develops [11].

The results of this study disagree with Venkataramana group who showed that all patients in their study were normoalbuminuric and without detectable micro- and macro vascular complications. In this study plasma total protein levels were statistically significantly increased [12].

3. Genomic level

Genotyping of the GSTM1 gene is GSTM1*A/A, GSTM1*B/B or GSTM1*A/B genotypes. But due to presence of a deletion in this gene, the possible genotypes would be GSTM1*A/A, GSTM1*A/0, GSTM1*B/B, GSTM1*B/0, GSTM1*A/B or GSTM1*0/0. PCR readings cannot differentiate between GSTM1*A/A, GSTM1*A/0 genotype, as both appear as positive band at the specific locus, are considered to be GSTM1*A wild genotype, while GSTM1*B/B and GSTM1*B/0 are considered as GSTM1*B mutant genotype [13].

In this study the result showed that the differentiation between GSTM1*A/A, GSTM1*A/0, GSTM1*B/B, GSTM1*B/0, GSTM1*A/B (represent positive results) in the hand and GSTM1*0/0(negative results) which called null or deletions by PCR.

Percentage of control group was 28% in Babylon community and this agreed with Seidgardet *al* who showed that total of 20 to 50% of persons had gene deletion, is called the GSTM1*0, or null allele

[6].Frequencies of GSTM1 homozygous null genotype (GSTM1*0) were 42%-60% in Caucasians and 16%-36% in Africans [14].

In this study, result shows that the largest percentage of deletion occur in metformin&glibinclidide group 68% who represented patients with type II diabetes mellitus that mean GSTM1*0 represent one of risk factor for type II diabetes mellitus and that is reaching agreement with Bhandari who proved that the GSTT1 and GSTM1 genes, alone or combined, have an influence on the risk of having type 2 diabetes mellitus [15] and Bidet *al* who demonstrated that the relationship of a joined impact of GSTM1, T1 and P1 genotypes in accomplice of Indian patients with T2DM. Since noteworthy affiliation was seen in GSTM1 invalid and GSTP1 and numerous relationship in GSTM1 invalid, T present and P1 (I/I), these polymorphisms can be screened in the populace to focus the diabetic danger [16].

The result of this thesis is disagreeing with Guoyinget *al* who showed that there was no association between either GSTM1 polymorphism and risk of T2 DM [17].The test displays that GSTM1*0 in insulin and insulin & metformin groups was the same 44% which were significant increase than present of control group and these groups represent type I DM that mean GSTM1*0 represent as risk factor for type I DM and that is agree with the result of Lynn, et al; who recommend that the GSTM1 invalid genotype is connected with T1D assurance and T1D age-at-onset and that helplessness to T1D may include GST conjugation [18], similarly Vojtkováet *al*,who stated that genotype combination GST T1 null/M1 wild was significantly more prevalent in subjects with diabetes and represented 2.9-fold risk for T1D developing [19].

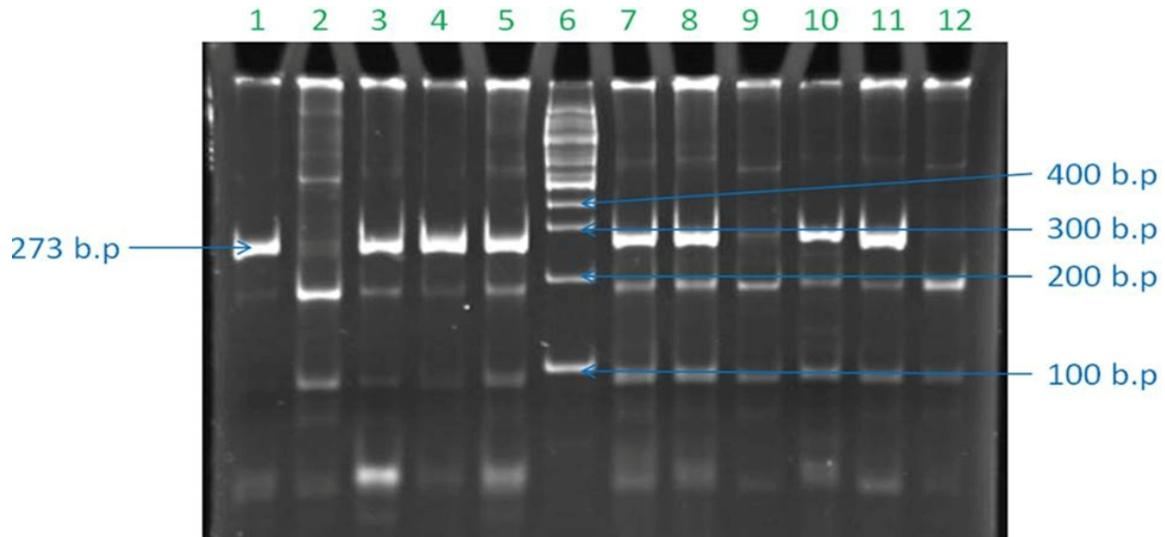


Figure shows the product of PCR in polyacryl amide gel 8% under UV light (1, 3,4,5,7,8,10 and 11) are normal GSTμ1. (2, 9, and 12) are deleted GSTμ1 6 is 100 b.p. DNA ladder.

Table 3: Association of Study Groups with GSTμ₁ deletion

Variable	Groups				P value
	Control (%)	Insulin & Metformin (%)	Metformin&Glibinclidimide(%)	Insulin (%)	
GSTμ ₁ Deletion	7 (28.0)	11 (44.0)	17 (68.0)	11 (44.0)	0.042 *
Normal	18 (72.0)	14 (56.0)	8 (32.0)	14 (56.0)	

*p value ≤ 0.05 is significant.

Deletion in the GSTM1 gene had a defensive part for diabetic retinopathy. A substantial number of studies on GSTM1-0 GSTT1-0 invalid genotypes report an expanded danger for improvement and movement of rheumatoid joint inflammation and asthma [20].

A large-scale cohort study in Egyptian population may confirm the role of GSTM1, T1 and P1 gene polymorphisms in the pathogenesis of T2DM and its related complications [21].

Significant correlation between GSTM1 null genotype and retinopathy could indicate this fact that impairs cellular metabolism result in increased free radicals and oxidative pressure. Therefore, GST null genotypes may result in decrease antioxidant capacity which causes side effects of diabetes [22].

Conclusions

Diabetic patient suffer from decrease in serum total protein may be due to increase excretion due to nephropathy or decrease in production due to defect in insulin secretion or action or both. From the result of glutathione S- transeferase μ 1 deletion may represent a highly risk factor for both types of diabetes mellitus.

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