Evaluating molecular study of the association of Glutathione S – Transferase GST (T1, M1) genetic polymorphism in Iraqi Arab Femals with Type 2 Diabetes Mellitus and Coronary Artery Disease

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Abstract:
Coronary artery disease (CAD) is a major health concern and leading of death in individuals with type 2 diabetes mellitus (T2DM). Glutathione S – Transferase(GST) are known for their broad range of detoxification and in the metabolism of xenobiotics . The role of functional variants of these genes in the development of various disorder is proven. We investigated the possible role of these variants in the development of CAD in T2DM patients. In this case – control study a total of 60 patients (T2DM = 30 ; T2DM – CAD = 30) and 30 controls were included. Serum lipid profiles were measured and DNA was extracted from the blood samples. Multiplex PCR for GSTT1/M1 (present / null) polymorphism, were performed for genotyping of study participants. Gene frequency and lipid profiles were statistically analyzed for disease association. Regression analysis showed that, there was no significant difference of the frequency of GSTT1 (positive /null) genotype and GSTM1 (positive /null) genotype in the 3 study groups . GSTT1 – positive genotype is associated with a 0.51 fold increased ( OR = 0.51 ; 95%CI = 2- 0.1 ;P = 0.321 ) , while the GSTM1 – positive genotype was associated with a 3 fold increase ( OR = 3.06 ; 95%CI=1- 9.7 ; P = 0.055) .We conclude GSTT1 positive genotype considered to be a protective risk from CAD in T2DM patients . The GSTM1 – positive genotype it was considered to be a risk factor of the CAD in T2DM patients.

Key words: Coronary Artery Disease, Type 2 Diabetes Mellitus, Glutathione S-Transferase, polymerase chain reaction.

Introduction:
Cardiovascular diseases are the leading cause of mortality among Type -2 diabetes mellitus (T2DM), which has a complex etiology that includes both atherogenic and myocardial components [1]. Almost 200 million people worldwide have Type 2 diabetes mellitus[2] and it is broadly recognized as an equivalent risk factor for coronary artery disease ( CAD)[3]. The prevalence, incidence and mortality of all cardiovascular disorders (CVD) are two – to eightfold higher in persons with diabetes than in those without diabetes [4] . Reactive oxygen species (ROS) production
induced by chronic hyperglycemia is implicated as a potential molecular mechanism behind diabetic vascular complications [5]. T2DM is associated with increased production of reactive oxygen species (ROS) and a reduction in antioxidant defenses leading to oxidative stress [6]. Oxidative stress, arising as a result of an imbalance between free radicals and antioxidant defenses, is associated with damage to lipids, proteins and nucleic acids, which could contribute diseases including atherosclerosis, cancer and diabetes mellitus [7].

Pancreatic β – cells have emerged as a putative target of oxidative stress induced tissue damage, and this seem to explain in part the progressive deterioration of β-cell function in type 2 diabetes mellitus [8].

Organisms have evolved many defense mechanisms as protection from these reactive intermediates (antioxidant). These include, but are not limited to, various enzymes such as glutathione S-transferase (GSTs), superoxide dismutase, Se-dependent glutathione peroxidase, catalase, glutaredoxins and peroxiredoxins [9]. Beta cells are very sensitive to cytotoxic stress because they express very little of the antioxidant enzymes. Hence, beta – cell is at greater risk of oxidative damage than other tissues with higher levels of antioxidant protection [10].

Glutathione (GSH) is the major cellular antioxidant that protects against environmental toxicant as well as reactive oxygen species (ROS) mediated injury. (GSH) detoxifies multiple compounds through glutathione S-transferase [11]. The glutathione S-transferase (GSTs) are gene super family enzyme that detoxify free radicals [12]. This plays an important role in protecting DNA against damage by genotoxins and adduct formation [13]. GSTs protects cells against oxidative stress [14]. GSTs are a family detoxification enzymes catalyze the conjugation of toxic and carcinogenic electrophonic molecular with glutathione and there by protect cellular macromolecules against toxic foreign chemicals and oxidative stress by free radical scavenging. Human GST enzymes are divided into three main families: cytosolic, mitochondrial and microsomal [15]. The enzyme active site is composed of two subsites, a G-site for binding GSH and an H–site for binding the hydrophobic electrophile. Based on, among others amino acid sequence identities, isoelectric points, substrate selectivities and immunological reactivities [16].

In human, glutathione S-transferase super family have been assigned to least eight separate classes designated alpha, mu, kappa, omega, pi sigma, theta and zeta, which are encoded by the GSTA, GSTM, GSTK, GSTO, GSTP, GSTS, GSTT and GSTZ genes, respectively [15].

GSTT1 and GSTM1 polymorphism are the most common polymorphism of the GST enzyme in the human population with major ethin differences [17]. GSTs have been found in many species including: humans, rate, bacteria, yeast, molds, insects, plants, fish and birds [18]. In human, the GSTM1 gene is situated in the GSTM cluster which has been localized to chromosome one in the region (1p13.3), five mu class genes are situated (GSTM1-GSTM5) on chromosome 1 [19]. The theta class of GSTs consists of two different subfamilies: GSTT1 and GSTT2. Gene encoding both proteins are located on chromosome (22q11.2) [20].

According to the reviewed literature, few studies have been published on the association between GSTT1/GSTM1 polymorphism and susceptibility to diabetes with coronary heart disease,
and there are large divergences among the study results. This first case – control study on the Iraqi population was designed to provide more information about the effects of the GSTT1 and GSTM1 polymorphism on T2DM and CAD risk. In this study obtained important results regarding the contribution of GST polymorphisms to T2DM with CAD and without CAD.

Subjects and methods

The study consisted of 60 patients (30 T2DM and 30 T2DM with CAD) and 30 health control women. Their age range was (40-65) years, from National – Diabetes Center, Al-Mustansiriya University.

Collection of blood samples:

From each patient groups and healthy human, (5ml) of blood was obtained by vein puncture using (5ml) disposable syringes after (12-14) hours fasting. The blood sample was divided into two aliquots: (3ml) and (2ml). The first aliquot (3ml) is dispensed in a plain test tube and left for around an hour to clot at room temperature, and then separated by centrifugation at (3000 rpm) for (10 min) to collect serum. The separated serum used for assays of lipid profile and fasting plasma glucose.

Genomic DNA extraction and genotyping:

Genomic DNA was isolated from (2 ml) whole blood collected in the EDTA tubes using the Wizard genomic DNA purification kit (Promega, USA)(Figure 1). Albumin as an internal control. PCR amplifications were performed in a total volume of 50μL containing 5μl genomic DNA, 14μL D.W., 25μL master mix and 1μL of each primer. as follows: GSTM1 were: 5' GAA CTC CCT GAA AAG CTAAG C 3', 5' GTT GGG CTC AAA TAT ACG GTG G 3'. The GSTT1 F: 5' TTC CTT ACT GTT CCT CAT GC 3' and GSTT1 R: TCA CCG CAT CAT GGC CAG CA 3'. The primers for albumin were Alb F:5' GCC CTC TGC TAA CAA GTC CTA C 3' and Alb R : 5' GCC CTA AAA AGA AAA TCG CCAATC 3'. Thermal cycling conditions includes: an initial denaturation step at 95°C for 3 min, 30 cycles at 94 °C for 1 min, 59 °C for 1 min and 72 °C for 1 min, and a final extension step at 72°C for 5 min.

The amplification products were size separated on 2% agarose gels and visualized by ethidium bromide staining (5%). GSTM1 and GSTT1 genotypes were determined by the presence and absence (null) of bands of 210 bp and 480 bp, respectively, with an internal control of 260 bp.

Using this genotyping assay of GSTM1 and GSTT1, the null genotypes can be clearly categorized, but the heterozygote and homozygote positive genotypes could not be differentiated [21] (Figure 2).

Fig. (1): Agrose electrophoresis of the genomic DNA sample. Fragments were fractionated by electrophoresis on 0.8% agarose (1h/70v), 1Xtb (tri-borate buffer) and visualized by ethidium bromide staining.
Fig. (2): A representative multiplex PCR analysis of GSTs polymorphism. GSTT1M and GSTT1 genes PCR products resolved by (2%) agarose gel electrophoresis (1h/70v). Lane M, DNA molecular weight marker. Lane B, negative control. Lane (1.13) is samples. A 350 pb DNA fragment corresponding to the albumin gene product provides an internal positive control for each reaction and can be seen in all PCR reaction. A 215 pb Is present only in those individuals containing the GSTM1 gene while a 480 pb products is present only in those individuals containing the GSTT1 gene.

Statistical analysis:
Data of continuous variables were expressed as mean ± Standard Deviation (SD) and data of non continuous variables as frequency (N%) .The data for three or more independent groups were evaluated by analysis of variance (ANOVA). The genetic variants and their risk for disease were computed by odd ratio (OR) and 95%CI Confidence Intervals by logistic regression analysis.

Results:
Table 1 shows the positive of the GSTT1 gene occurs higher frequencies in healthy controls 26(86.7%), than patients T2DM without CAD 25( 86.2%) and patients T2DM with CAD 23(76.7% ) , p= 0.51 there was no significant difference between three study groups .

While the GSTM1 shows the higher frequencies in T2DM with CAD 13(43.3%), than patients T2DM without CAD 9(31%) and healthy control 6(20%). There was no significant difference between them.

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<th>Table 1: Comparison of GST genotypes among three population</th>
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Significant difference p>0.05 , non significant p<0.05

Association of GST polymorphism with T2DM and CAD
In the (table 2) showed the risk estimates for CAD among the diabetic patients .GSTT1 positive genotype was associated with 0.66 but the GSTM1 positive genotype was associated with 1.78 – fold . There was no significant difference between them .There was no risk found for (GSTT1and GSTM1) null genotypes. We observed that the GSTT1 positive genotype was considered to be a protective risk because of the rate of OR = less than one.

But the GSTM1 positive genotype was considered a risk factor to development the CAD in T2DM patients because of the rate of OR = more than one.

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<th>Table 2) The risk of having T2DM with CAD in the presence of two genes compared to cases having T2DM without CAD.</th>
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OR – odds ratio and CI – confidence interval from conditional logistic regression analysis.
In Table 3, the distribution analysis for the both GSTT1 and GSTM1 genotypes can be observed. There was a low frequency of individuals who had a double null genotype (-/-), for both T2DM without CAD patients and T2DM with CAD patients (10.3% and 6.7%, respectively).

A higher prevalence of individuals with a GSTT1 present / GSTM1 null for both groups (58.6% and 50.0%, respectively) was also observed.

**Discussion:**

Diabetes Mellitus is one of the most common chronic diseases in nearly all countries, the number of people with diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and reduced physical activity [22]. T2DM is a major risk factor for coronary artery disease (CAD) resulting in high morbidity and mortality [23]. Imbalance between free radicals and anti-oxidant defenses is associated with cellular dysfunctions leading to the pathophysiology of various diseases [7]. Experimental evidence suggests that diabetes is associated with a reduced overall antioxidant defense system [24] and the increased oxidative stress may contribute to the pathogenesis of the diabetic complications, notably the emergence of premature atherosclerosis [25]. The role of oxidative stress in the development of coronary artery disease is well known [26].

Our study showed there was no significant difference between three study groups which agree with Hussain et al. (2012) who found there was no significant difference between GSTT1(positive/null), P= 0.157 as well as between GSTM1(positive/null), P= 0.360 in patients and controls. While Ramprasath et al., (2011) who found the GSTT1 positive genotype occurs at higher frequencies in controls than in T2DM with CAD and T2DM without CAD patients, but the GSTM1 positive genotype occurs at higher frequencies in controls than in T2DM with CAD patients and T2DM without CAD patients, there were significant difference between three study groups in two status of GSTs (T1,M1) with CAD.

This study showed the distribution of GST genotype and alleles of the patients groups were given, the GSTT1 positive genotype occurs at higher frequencies in T2DM without CAD than in T2DM with CAD, while the GSTM1 positive genotype occurs at higher frequencies in T2DM with CAD patients than in T2DM without CAD patients.

Previous studies regarding the association between GST polymorphism and cardiovascular diseases have also shown conflicting results, with striking differences between ethnic groups [27,28]. These results which disagreement with Pinheiro et al., (2013) who found that the GSTT1 null genotype (p = 0.0004) is related to an increased predisposition for T2DM, conferring a 3.2 fold increased risk of developing the disease relative to the present genotype. It was also observed that there wasn’t any association of the GSTM1 null genotype with
susceptibility to disease in the population studied (OR = 1.1, 95%CI = 0.66-1.82, P = 0.732). On the contrary, similar study in the Turkish population reported that both GSTT1 and GSTM1 null genotype were associated with increased coronary artery disease, which became statistically significant only with smoking [27]. Another Indian study has complicated it further, reporting that the GSTT1 null genotype is protective against coronary artery disease [28]. Given all these conflicting results, one might think that the detoxification capacity of oxidative stress does not simply parallel the lowering of cardiovascular risks.

Our results are lower frequency of individuals who had a double null genotype (–/-) , for both patient groups (T2DM without CAD and T2DM with CAD) was (10.3%) and (6.7%) respectively . A higher prevalence of individuals with a GSTT1 present /GSTM1 null for both groups (58.6%)/(50.0%) respectively . According to these results we consider the (GSTM1+) genotype a risk factors to developed the CAD in T2DM patients. These results agreement with some researches which reported that the GSTT1 genotype reduces the incidence rate of type 2 diabetes mellitus and might be a protective factor[29,30]. This may be the first study of association between T2DM, CAD and GSTs polymorphism in Iraqi population . Our results found that the GSTT1(+) genotype was protective factor for having T2DM or CAD while GSTM1 positive genotype was a risk factor for T2DM and CAD by increasing 1.78 fold. This finding didn't agreement with other studies.

It is difficult to determine the role of polymorphism of different proteins involved in the inflammation in CAD . The studies of this kind have limitation in establishing the causal role in the disease. However, these studies contribute to the scientific evidence in elucidating the etiolo of complex multifactorial diseases like CAD and hopefully provide a step in the search for candidate genes.

Conclusion: Our results suggest that GSTM1 gene polymorphisms may play an important role to development CAD in T2DM patients.

References:


تقييم الدراسة الجزيئية للعلاقة بين الجلوتاثيون آس – ترانسفيريز (T1,M1) في الإناث العراقيات العربيات مصابات بداء السكري من النوع الثاني و امراض الشرايين القلبية الناجمة

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الخلاصة:
مرض الشرايين القلبية الناجم عن المخاطر الصحية الخطرة التي تؤدي إلى وفاة الأشخاص المصابين بداء السكري من النوع الثاني. أنزيم الجلوتاثيون آس – ترانسفيريز واسع المدى في إزالة السمية والاستقلاب الإيسي، تثبت دور العوامل الزائدة من هذه الجينات في تطور مختلف الأضطرابات المرضية. لذا، فإن هذه الدراسة تبحث عن دور المحتمل لهذه العوامل في تطور إمراض الشرايين القلبية في المرضى الذين يعانون من نوع الثاني. في هذه الحالة، تم دراسة على مجموعة من 60 مريض، من مرضى السكري فقط و 60 مريض مصاب بالسكري و أمراض القلب، و 300 من الإناث الصحية. تم قياس الندهون الحمضية و عدم استخلاص الدنا DNA من عينات الدم و إكمال PCR للكشف عن جينات GSTT1 و GSTM1. و اظهرت التحليلات الإحصائية بطول وجود اختلافات معنوية بين المجاميع الثلاثة، بالإضافة إلى وجود أو عدم وجود الجينات. أظهرت التحليلات الإحصائية بان وجود الجين GSTT1 يرتبط مع عدالة 0.51 وهذا يدل على أن وجود الجين 1 OR=3.6، بينما وجود الجين GSTM1 يرتبط مع عدالة 0.51 OR=3.6، وهذا يدل على أن وجود الجين 1 OR=3.6، بينما وجود الجين GSTM1 يرتبط مع عدالة 0.51 OR=3.6، وهذا يدل على أن وجود الجين 1 OR=3.6. يعتبر عامل خطر للإصابة بالأمراض القلبية في الأشخاص المصابين بداء السكري من النوع الثاني.

الكلمات المفتاحية: مرض الشرايين القلبية الناجم، مرض السكري من النوع الثاني، جلوتاثيون S- ترانسفيريز، تفاعل السلسلة المتعددة.

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