Glutathione S-Transferase Gene with Susceptibility to Juvenile Idiopathic Arthritis

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Abstract

Introduction: Juvenile idiopathic arthritis (JIA) is a chronic autoimmune disease of unclear etiology. It is the most common inflammatory arthritis worldwide with major individual and health service coast. GSTs play important roles in detoxification mechanisms. It is known to be polymorphic and the presence of polymorphisms has been implicated in susceptibility of JIA.

Aim: The aim of this study was to explore the association between GST gene and susceptibility to JIA.

Subject& methods: 40 patients with JIA and 40 apparently healthy controls matched with age and sex were genotyped using allelic discrimination by PCR.

Results: the frequency of GSTM1 null genotype polymorphism was significantly higher in JIA patients than in controls (OR= 0.4, CI= 0.16-0.98, P= 0.04). No significant association was found regarding GSTT1 null gene polymorphisms in JIA patients (OR= 0.417, CI= 0.15- 1.13, P= 0.8). GSTM1null gene polymorphism is more prevalent in females than males (P= 0.004), whereas no significant association was found in GSTT1 gene polymorphism (p= 0.4).

Conclusion: Higher frequency of GSTM1 null genotype polymorphism in patients of JIA suggesting that it might be associated with susceptibility of JIA, severity and outcome. GSTT1 null gene polymorphism had no association with JIA susceptibility.

Key words: Juvenile idiopathic arthritis, Glutathione- S- Transferase, GSTM1, GSTT1, Polymorphisms.

جين الجلوتاثيون والاستعداد للإصابة بالروماتويد فى الأطفال

المقدمه: يعد مرض الالتهاب المفصلى الحدثى مجهول السبب واحداً من أكثر الأمراض الرماتيزمية شيوعاً فى الأطفال، كما يعد من أهم أسباب الإعاقة سواء على المدى القريب أو البعيد. ومازالت حتى الآن الأسباب وراء هذا المرض غير معلومة بصورة واضحة حيث أن هناك العديد من الأسباب الوراثية المركبة التى تتضمن تأثير العديد من الجينات المتعلقة بالمناعة والالتهاب. يعد الجلوتاثيون من أهم الإنزيمات التى تحمى الخلية ضد السموم المنتجة كيميائياً إما مباشرةً أو بالإتحاد الإنزيمى (إنزيم تحفيز إنتقال الجلوتاثيون) وعناصر الأوكسجين التفاعلية وتنتج بيرأوكسيدات دهون الغشاء عن توليد مجموعة حرة أو نقص فى أنشطة أنظمة مضادات الأكسدة الدفاعية. الجلوتاثيون معروف إنه متعدد الأليل ولهذا فهو متورط بمرض الالتهاب المفصلى الحدثى مجهول السبب ولهذا فإن دراسة التنوعات الجينية للجلوتاثيون تستطيع أن تساعد فى فهم التنوعات الفردية للقابلية للإصابة بالمرض ولهذا كان الهدف من هذة الدراسة توضيح دور التنوعات الجينية للجلوتاثيون والقابلية للإصابة بمرض الالتهاب المفصلى الحدثى مجهول السبب.

الهدف: أجريت هذه الدراسة فى عيادة الحساسية والمناعة للأطفال بمستشفى الأطفال الجامعى بجامعة عين شمس. من هذه الدراسة نستخلص أن التنوعات الجينية للجلوتاثيون من الممكن أن تلعب دورا هاما وحيويا فى القابلية للإصابة بمرض الالتهاب المفصلى الحدثى مجهول السبب.

النتائج: أن نسبة التنوع الجينى العدمى فىGSTM1 كانت أعلى فى مجموعة المرضى عن المجموعه الضابطة وكانت مدلول إحصائي، ونسبة التنوع الجينى الغير عدمى فى GSTM1 كانت أعلى فى المجموعة الضابطة عن مجموعة المرضي، ونسبة التنوع الجينى العدمى فى GSTT1 كانت أعلى فى مجموعة المرضى عن المجموعه الضابطة، ونسبة التنوع الجينى الغير عدمى فى GSTT1 كانت أعلى فى المجموعة الضابطة عن مجموعة المرضي.

الخلاصه: نستخلص أن التنوعات الجينية للجلوتاثيون من الممكن أن تلعب دورا هاما وحيويا فى القابلية للإصابة بمرض الالتهاب المفصلى الحدثى مجهول السبب.

الكلمات الكاشفة: الالتهاب المفصلى الحدثى مجهول السبب. الجلوتاثيون. التعدد الأليلي.

Introduction:

Juvenile idiopathic arthritis (JIA) is one of the most common rheumatic disease of children and a major cause of disability. It is characterized by an idiopathic syonvitis of peripheral joints associated with soft tissue swelling and effusion (Miller and Cassidy, 2004). The disease primarily affect the joint but can also cause heavy damage to organs and systems such as the heart, blood vessels, skin, eyes, and peripheral nerves (Meholjic- Fetahovic, 2005). Current slow- acting anti rheumatic drugs (SAARDs) have limited efficacy and many side effects. Moreover, they do not improve the long- term prognosis of the disease (Loetscher and Moser, 2002). GSTs enzymes constitute a family of cytosolic isoenzymes that are involved in the detoxification of electrophilic xenobiotics. They represent an important group of enzymes which detoxify both endogenous compounds and foreign chemicals such as pharmaceuticals and environmental pollutant (Townsend and Tew., 2003). Numerous polymorphisms exist in the human GSTs genes, leading to decreased detoxification of environmental carcinogens or chemotherapeutic agents and thus to clinical problems in patients lacking these genes. Several studies have demonstrated that multiple allelic polymorphisms at loci encoding detoxifying enzymes are the basis of inter-individual variation in detoxification metabolism. Differences in genetic susceptibility to diseases can be partly attributed to inter- individual variation in metabolic activity (Cotton et.al., 2000 and Zhang et.al., 2011). Genetic polymorphisms of these enzymes may contribute to the wide variation seen in the extent of joint damage and functional impairment (Yun et.al., 2013).

In view of such data, this study is carried out to explore the association between GST genes and susceptibility to JIA.

Subjects And Methods:

This case- control study was conducted on 80 neonates; group A, included 40 JIA patients (13 male and 27 female) and group B, included 40 apparently healthy children (18 male and 22 female) serving as controls. The two groups were age and sex matched. The study was carried out in pediatric allergy and immunology clinic Ain Shams University over the period from June 2011 to March 2012. The study protocol was approved by the ethics committee of Institute of Postgraduate Childhood Studies, Ain Shams University. Written informed consent was obtained from the parents. The inclusion criteria for the JIA group were: all patients were diagnosed before 16 years of age, presented with inflammatory arthritis in at least one joint and fulfilled the JIA criteria. On the other hand, patients with inflammatory pathologies such as connective tissue disorders, ulcerative colitis, diabetes or asthma. And patients with malignancy were excluded.

The studied patients and controls were subjected to thorough history taking, full clinical examination, laboratory study including CBC, ESR, CRP, RF, ANA and imaging study for patients only. Molecular study of GSTM1and GSTT1 polymorphisms was carried out using allelic discrimination by PCR.

Sample Collection:

Paired EDITA blood and serum were collected from patients and controls using Vacutainer system.

Molecular analysis and genotyping of GSTM1 and GSTT1:

- DNA isolation: was done under complete sterile condition in a biosafety. Genomic DNA was isolated from EDTA- peripheral blood using Mini- Spin- Column protocol (Qiagen, USA) as recommended by the manufacturer. Proteinase K (20 µl) was pipetted into the bottom of a 1.5 ml- micro centrifuge tube. A whole blood sample (200- µl) was added to the micro centrifuge tube. We use up to 200 µl whole blood, plasma, serum, buffy coat, or body fluids, or up to 5x106 lymphocytes in 200 µl phosphate- buffered saline (PBS). Lysis buffer (AL) (200 µl) was added to the sample and incubated at 56°C for 10 min for complete hemolysis of the RBCs, lysis of the WBCs pellet and digestion of the proteins. Absolute ethanol (200- µl) was mixed with the sample to precipitate the DNA. The sample- ethanol was carefully applied to the QIAamp spin column (QIAGEN, USA),a nd then the mixture was centrifuged at 8000 rpm for 1 min. The filtrate was discarded. The column was carefully washed with the buffered solution (AW1) (500- µl). The tube was centrifuged at 8000 rpm. Another 500- µl of the Washing buffer (AW2) was added and repeat centrifugation at full speed for 2 min. The column was opened and 200- µl Buffer AE (Elution buffer) were added, incubated at room temperature for 1 min, and then centrifuged at full- speed for 1 min. The highly pure DNA sample was refrigerated at 4°C till use, - 20°C for longer time or- 70°C forever.
- Genotyping of GSTT1 and GSTM1 genes: were performed by multiplex polymerase chain reaction (PCR). GSTM1, GSTT1 and B-globin in genes were simultaneously amplified by PCR with mixed primers for each gene. Primers were as follows: GSTM1 Sense 5'GAACTCCCTGAAAAGCTAAAGC-3'. Anti sense 5'-TTGGGCTCAAATATACGGTGG-3' GSTT1Sense 5'-TTCCTCACTGGTCCTCACATCTA-3' Antisense 5'-TCACCGGATCATGGCCAGCA-3' B-globin Sense 5'-GAAGAGCCAAGGACAGGTAC-3' Anti sense 5'-CAACTTCATCCACGTTCACC-3' B-globin gene was used as internal control. PCR was carried out in a total volume of 50µl containing 0.25 mm dntp, 1.5 units taq polymerase (Invitrogen, corporation, San Diego, CA, USA), 5 pmoles of each primer and 300 ng sample DNA. The amplification protocol consisted of: Initial denaturation at 94 for 4 minutes. Followed by 35 cycles of denaturating (94°C for 1 minute), annealing (55°C for 45 seconds), and extension (75°C for 1 minute). Final elongation at 72°C for 7 minutes. This protocol was carried out using a thermal cycler. PCR products were separated by 2.5% agarose gel electrophoresis containing 1 ug/ml ethidium bromide, and visualized under an ultraviolet transilluminatior. The expected sizes of amplified products of GSTM1, GSTT1, and B- globin were 219 base pairs, 480 6p, and

268 bp, respectively.

Statistical Analysis:

Data was analyzed using (SPSS) version 18.0 (SPSS Inc., Chicago, IL, USA). Descriptive Statistics in the form of mean (X) and standard deviation (SD) were performed for all patients. For quantitative data, Student t- test (t) was used. For comparing qualitative data, Chi square test (X2), Odd ratio (OR) and confidence interval (CI) where applied. Values of P <0.05 were considered statistically significant; values were highly significant if <0.001 and the OR was significant if > 1.

Results:

The characteristics age, sex and type of onset in JIA cases and controls enrolled in this study were shown in table (1) which shows that the female patients were more than male patients and the most common type of JIA was polyarticular. (47.5%), followed by systemic onset type (30%) then pauciarticular type (22.5%).

Table (2) show that the profile of JIA cases, comparing the type of onset with age of onset, duration of illness, number of tender joints and number of swollen joints. Quantitative data are expressed as mean and standard in years and no statistical significant difference was found.

Comparing family history among the studied group was shown in table (3) with significant difference between the three subtypes (P= 0.01). The highest frequency of +ve family history was in polyarticular type (55.6%) while the systemic type has no +ve family history.

Table (4) shows the genotypic analysis of GSTM1 between JIA cases and controls, in which a significant difference in the genotypic analysis of GSTM1 between JIA cases and controls (OR= 0.4, CI= 0.16- 0.98, P= 0.04) was found. The null frequency GSTM1 genotypes, was higher in cases than controls (62.5% vs 40%). While the frequency of non- null genotypes were higher in controls than cases (60% vs 37.5%).

Table (5) shows genotypic analysis of GSTT1 between cases and controls of JIA in which no statistically significant difference in the genotypic analysis of GSTT1 between JIA cases and controls (OR= 0.417, CI= 0.15- 1.13, P= 0.8). The null frequency GSTT1 genotypes of was higher in cases than controls (37.5% vs 20%), while the frequency of non-null genotype was higher in controls than cases (80% vs 62.5%).

Table (6) show the genotyping of GSTM1 and sex in the studied group in which the frequencies of the null genotype were more prevalent in females versus males (84% vs 16%) with statistically significant difference (P= 0.004).

Table (1) The characteristics age, sex and types of onset in JIA cases and controls enrolled in this study

Controls (N= 40)	Patients No (N= 40)	Ch. Ch	
8.46±3.77	9.25±4.55	Age (Mean±Sd)/Years	
18 (45)	13 (32.5)	Male N (%)	Sex
22 (55)	27 (67.5)	Female N (%)	
	19 (47.5)	Polyarticular	Types Of Onset
	9 (22.5)	Pauciarticular	
	12 (30)	Systemic	

Table (2) Profile of JIA cases, comparing the type of onset with age of onset, duration of illness, number of tender joints and number of swollen joints.

Variable	Pauci Articular	Poly Articular	Systemic	F	P- Value
Age Of Onset Mean±Sd/Years	5.63±3.71	6.24±3.72	5.38±3.75	0.21	0.8
Duration Of Illness/Years	4.36±3.88	2.51±1.72	4.31±4.26	1.63	0.2
Number Of Tender Joints	5.11±2.52	6±3.35	5±2.59	0.51	0.6
Number Of Swollen Joints	4.22±2.72	3.74±2.72	2.67±1.3	1.22	0.3

Table (3) Distribution of family history among the JIA studied group.

Family History	Pauci Articular N (%)	Poly Articular N (%)	Systemic N (%)	X^2	P- Value
+Ve	4 (44.4)	5 (55.6)	0 (0)	8.38	0.01
- Ve	5 (16.1)	14 (45.2)	12 (38.7)		

Table (4) Genotypic analysis of GSTM1 between JIA cases and controls.

	GSTM1		X^2	P- Value	OR (95%CI)
	Non- Null N (%)	Null N (%)			
Cases	15 (37.5)	25 (62.5)	4.05	0.04	0.4 (0.16- 0.98)
Controls	24 (60)	16 (40)			

Table (5) Genotypic analysis of GSTT1 between cases and controls of JIA

	GSTT1		X^2	P- Value	OR (95%CI)
	Non- Null N (%)	Null N (%)			
Cases	25 (62.5)	15 (37.5)	2.9	0.8	0.417 (0.15- 1.13)
Controls	32 (80)	8 (20)			

Table (6) Genotyping GSTM1 and sex in the studied group.

Sex	GSTM1		X^2	P- Value
	Non- Null N (%)	Null N (%)		
Male	9 (60)	4 (16)	8.27	0.004
Female	6 (40)	21 (84)		

Discussion:

JIA is a chronic autoimmune disease of unclear etiology, it is evidently shown that the expression and development of the disease is due to combination of genetic and environmental factors, moreover, the onset is likely to involve multiple genes (LI et.al., 2009).

JIA is the most common inflammatory arthritis worldwide with major individual and health service coast and characterized mostly by polyarticular inflammation, increased cytokine production and pannus development, which subsequently lead to the erosion of the cartilage and underlying bone (Song et.al., 2012).

ROS are involved in JIA pathology, since they are generated by neutrophils, monocytes and macrophages in synovial fluid of inflamed joints and cause DNA and lipid oxidation leading to cartilage and bone destruction. The defense mechanism against ROS is complex and involves several enzymes including GSTs (Graber et.al., 2009).

GSTs are a widely expressed supergene family encoding bio transforming dimeric enzymes that catalyse the conjugation of glutathione and are implicated in the detoxification of free radicals and prostaglandins. They have a peroxidase activity towards cytotoxic secondary metabolites, thus have an important role in cellular protection

against ROS (Chen et.al., 2012).

Polymorphisms in GST genes have been described in several studies; some of them alter enzymatic activity and may modify the ability for the elimination of ROS products. Polymorphisms associated with reduced GST activity, most commonly GSTM1 and GSTT1, can help understanding individual variability in the susceptibility to the development of JIA (Ji and Lee, 2013).

Accordingly this study is carried out to explore the association between GST genes and susceptibility to JIA.

The results of the present study showed a statistical significant difference comparing family history and the three subtypes of the studied group. The highest frequency of +ve family history was in polyarticular type (55.6%) while the systemic type has no +ve family history. Family history of JIA will remain an important risk factor for JIA. Similarly, Prahald et.al. (2002) reported that the prevalence of autoimmunity is significantly higher among first and second degree relatives of JIA. This suggested that clinically different autoimmune phenotypes may share common susceptibility gene which may act as risk factor for autoimmunity.

In the current study, as regard GSTM1 genotypes, the null frequency was higher in JIA patients than controls (62.5% vs 40%). However the frequency of non- null genotypes were higher in controls than patients (60% vs 37.5%) with a statistical significant difference.

Yun et.al. (2005) and Morinobu et.al. (2006) reported that the frequency of GSTM1 null genotype was significantly higher among patients than controls, and the functional allele for GSTM1 may reduce risk of JIA thus, GSTM1- null polymorphism is associated with increased susceptibility and severity of JIA. Moreover, Rohr et.al. (2008) stated that GST genes polymorphism seem to interfere with disease susceptibility, outcome and response to treatment.

However, Keenan et.al. (2010), Ghelani et.al. (2011) and Mikuls et.al. (2012) stated that no association was found between GSTM1 genotype and JIA susceptibility. These discrepancies might be due to ethnic specific genetic variations, which could greatly influence susceptibility to the disease. Another possible explanation might be due to different genetic and environmental backgrounds among populations/the relative small number of the studied population.

In the present study, no statistical significant difference in the genotypic analysis of GSTT1 between JIA patients and controls. As regard GSTT1 genotypes, the null frequency was higher in patients than controls (37.5% vs 20%), while the frequency of non- null genotype was higher in controls than patients (80% vs 62.5%). Similarly, Yun et.al. (2005), Morinobu et.al. (2006), Ghelani et.al. (2011), Song et.al. (2012) and Ji and Lee (2013) stated that no association was found between GSTT1 null genotype and JIA.

In contrast to the results of this study, Mattey et.al. (2000) and Rohr et.al. (2008) had reported that higher frequency of GSTT1 null genotype was observed in JIA patients, suggesting that GST enzyme are involved in JIA susceptibility.

The present study revealed the frequencies of the GSTM1 null genotype were more prevalent in females than males of JIA patients (84% vs 16%) with statistically significant difference. Moreover, Morinobu et.al. (2006), stated that the GSTM1 null genotype is associated with disease susceptibility in females, but not in males. This may be due to small population of the male patients in the study. Another possible explanation is that the GST genes may have different effect on disease susceptibility in male and female patients because of the polygenic nature and the sex-related predisposing factors of the disease.

In conclusion, the GSTM1 null genotype could be a genetic factor that determines susceptibility to JIA and may have influence on the disease process. GSTT1 null genotype is not associated with an increased susceptibility to JIA. GST gene polymorphisms might be crucial factors, which may serve as biomarkers of protection or susceptibility of JIA.

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