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Association of some genetic polymorphisms of heat shock 90 with type 2 diabetes and complications of diabetic foot

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Summary

This study was conducted in the Department of Biology, College of Science, Al-Qadisiyah University, and Diabetes and Endocrinology Center in Al-Qadsiyah Governorate. For the period between the July month of 2022 to the February month of 2023, 200 blood samples were collected from volunteer patients and visitors to the Diabetes Center of both sexes with different ages ranging between (45-70) and of both sexes in Diwaniyah Governorate. Fifty samples were used for each group, and the study design consisted of Two groups divided between the first group (G1) the control group and group (G2)Type 2 diabetes with diabetic foot) In this study, the concentration of heat shock protein 90 and Polymorphism of the Q488H gene, . Significant in the concentration of heat shock protein 90 in the injured groups and a significant difference with a probability level ($P \leq 0.05$. The polymorphism of the Q488H gene (G>C) amplified using the ARMS-PCR technique, amplification refractory mutation system, showed a higher percentage of the G allele in the infected sample compared to the C allele, and the G allele appeared as a causative allele associated with the risk of developing the disease, while the C allele recorded a higher percentage Of the G allele in the standard sample, the C allele appeared as a protective allele against disease. The genotypes CC and CG appeared as two patterns associated with the risk of developing diabetic foot caused by type 2 diabetes, and the genotype GG appeared as a genotype associated with the risk of developing diabetic foot caused by type 2 diabetes.

Keywords: Diabetes typy2, polymorphism, Q488H,FootUlcer .

1-Introduction

Heat shock protein HSP90 plays a major role in stimulating cell proteins, as HSP90 had a role in stimulating different biological processes, especially coordinating regulatory mechanisms in order to control the activity of these processes, and HSF1 which is the heat shock factor had a role in regulating HSP90, HSF1 integrates biological signals to regulate HSP90 levels, especially during times of stress, and not only regulates heat shock protein 90 chaperones, but also releases HSP90 from pancreatic beta cells when exposed to inflammatory conditions(Pinuinti et al ., 2019).

Molecular heat shock protein (HSP90) assists the abnormal protein becoming as well as stabilizing unfolded proteins. There are proteins in the cell that depend on HSP90 in function to HSPs90 as well as regulation of ATPase in HSP90 and stabilization of the different conformational states in HSP90. Proteins that are molecular chaperones with HSP90 are essential for the development of Various diseases, including cancer, Alzheimer's, neurodegenerative diseases, as well as viral and bacterial infections, and as a result of these relationships between chaperones and HSP90, it was found that targeting HSP90 and partial chaperones is an effective way to combat a wide range of diseases. (GA) and radial (RD), the interaction of HSP90 with (GA) led to a marked decrease in the growth of cancer cells and a decrease in tumor-causing proteins (Abbey , et al.,2017).

Chronic ulceration of the skin in diabetic patients is a painful condition that affects patients with diabetes, which constitutes 15% of patients with this disease worldwide. Heat shock protein (HSP90) is a stimulator (iNos) Nitric oxide synthase and vascular endothelial growth factor vascular endothelial grout factor (VEGF) in the skin of diabetic laboratory animals, where the expressions of HSP90, (ions) and (VEGF) in the skin were more concentrated in the experimental animals with diabetes than in the control animals, where the results indicated that diabetes became stimulatory Stabilization of expressions of VEGF, inos, and HSP90 in skin tissues of diabetic animals and may affect skin wound healing (Khalid et al., 2021).

Diabetes mellitus is a disease of the age, and it is in fact many physiological imbalances, which are characterized by an excessive amount of sugar in the blood, which is directly caused by insulin resistance or the lack of insulin secretion in a normal or sufficient amount. Lead to the destruction of insulin-producing cells in

the pancreas organ. Type 2 diabetes nowadays is more common and its problem lies in the gradual regulation of glucose due to damage to beta cells as well as insulin resistance (Blair., 2016).

Diabetic foot ulcer (DFU) are a major source of preventable morbidity in adults with diabetes. Consequences of foot ulcers include functional deterioration, infection, hospitalization, lower limb amputation, and death. The lifetime risk of developing a foot ulcer ranges from 19% to 34%, and this number increases with increasing longevity and medical complexity for people with diabetes. Morbidity following incident ulceration is high, with recurrence rates of up to 65% at age 3–5 years, a lifetime incidence of lower extremity amputations of 20%, and a 5-year mortality rate of 50–70%. New studies indicate that the incidence of total amputation has increased by up to 50% in some areas over the past several years. After a long period of regression to diabetes mellitus, DFU is a common and highly pathological complication of diabetes. The pathway to ulceration, which includes loss of sensation, ischemia, and minor trauma, is well established. Amputation and mortality after DFU represent late-stage complications and are strongly associated with poor diabetes management. Prevention and early detection of DFU through guideline-guided multidisciplinary care is critical to reducing complications of diabetes mellitus(Katherine et al .,2023). Diabetic foot ulcer is often one of the most important complications of diabetes, including its cost and destructiveness to health, and there must be control of blood sugar and maintaining it in good health accompanied by dressing for wounds (Leila et al., 2015). Hsp90 (heat shock protein 90) is a molecular helper responsible for the activation and maturation of the proteins produced. Surprisingly, Hsp90 appears to remodel newly produced proteins, acting as a vehicle by which the structure of a protein is modified to allow its subsequent remodeling into an active state, in the case of kinases, or by rendering the product protein eligible to bind to hormones, as in the case of the GR (receptor) glucocorticoids, Such as CDK4 (cyclin-dependent kinase 4) and GR, which are activated according to the so-called “remodeling hypothesis” for their activation. A detailed description of these activation mechanisms is critical to understanding how Hsp90-related diseases develop. (Chrisostomos .,2022).

1-1Amplification refractory mutation system (ARMS-PCR)

ARMS-PCR: Thermal Mutation Resistant to Amplification of Quadruple Primer PCR System (ARMS-PCR) is a multiplex type PCR, which provides rapid and fast assays for SNP analysis (locus stands for nucleotide)coupled to sequencing or soluble point analysis by Combinations of two allele-specific exonic and two intrinsic primers require bar coding (i.e., genotyping) only standardized PCR and isolation of fragments by electrophoresis.(A.M. Alzohairy et al .,2015).

ARMS (thermal amplification mutagenesis system), which allows genotyping only by examining reaction mixtures after agarose gel electrophoresis. The system is simple, reliable and accurately detects allele mutation. It will clearly distinguish heterozygous at a locus from homozygous for either allele. The system does not require restriction enzyme digestion, or specific oligonucleotides as they are traditionally known, and no sequence analysis of PCR products occurs. The basis of the invention is that, unexpectedly, oligonucleotides with mismatched 3′-residues will not function as primers in PCR under the appropriate conditions.(C.R. Newton et al.,1989). The discovery of the ARMS-PCR technique is of great importance in genetic studies and is considered one of the preferred technologies in terms of detecting the presence of the mutation or the genotype of the mutation clearly, and the method of using it is easy so that it is more appropriate in terms of analyzing a large number of samples in genetic research, and in terms of its high health rate, It is important to mention that the ease of use of the output and analysis of base sequences in relation to the genetic analysis device. (Duta et al., 2009).

3 -Materials and Methods

3-1Experiment design

One hundred individuals with age ranged between (45-70) years were enrolled in this study. They divided into -:three groups as follows:

1.-Group (G1) that consists of 50 healthy individuals as control group.

The patients attended the diabetic and 2-Group (G2) that consists of 50 diabetic foot ulcer patients endocrinology center in Al- diwanya Teaching Hospital of the Iraqi Ministry of Health ,during period July 2022 to February 2023. Patients with smoking and kidney(without Diabetes) disease were excluded. The polymorphism of the 90 HSPs of the mutant gene (C>G) Q488H was determined using ARMS-PCR as follows:

3-2Sample Collection

5 milliliters of venous blood was drawn from the study cases and control sample placed in a plane tube and left for (15 min) at room temperature, and then centrifuged at 3500 rpm for 10 min. Serum that obtained was stored at (-20oC) unless used immediately. whole blood was used in the a studied of polymorphism of gene Q488H.Samples were collected from the patient of DM2 From and Endocrinology Center at Al-Diwaniyah Teaching Hospital (5-6 ML) of venous blood was drawn by medical syringe. (3ML) of venous blood of patient was withdrawn by a single-use medical syringe and then placed in special anticoagulant EDTA tubes for the purpose of conducting Molecular tests.

3-3 Statistical analysis

The data were analyzed using the statistical program. Statistical Package for Social Sciences ver. 22 (SPSS), and significant differences between the means were compared using Mann-Whitney U tests. Fisher's and Fisher under the probability level. Under the probability level of $P \leq 0.05$, genotypes and their alleles and the critical ratio (OR) were analyzed. Hardy-Weinberg equilibrium and according to the program available on the website www.had2know.com/academics.htmI. The results of the DNA sequencing study were analyzed using Chromas Pro software manufactured by the company ChromasPro Technelysium Pty Ltd (version 1.6, 2012) and software(Hall., 1999).

4- Results & Discussion.

Results and discussion of the molecular study:

Results and discussion of the molecular aspect polymorphism of the Q488H gene for heat shock proteins 90 in the blood serum of the studied samples.The polymorphism of the 90 HSPs of the mutant gene (C>G) Q488H was determined using ARMS-PCR as follows:

The results of the electrophoresis of the amplified mutant gene (C>G) using ARMS-PCR technology indicated the presence of two alleles, the C allele and the G allele, and the presence of two genotypes, GG and GC, in all studied samples with type 2 diabetes, while three genotypes, CC, appeared in the standard sample , CG, GG as shown in the two) fig (2-4) , (1-4)

Genetic results of with type 2 diabetes.

The results of the frequency distribution of the G and C alleles of the Q488H gene, using the Hardy-Weinberg equilibriumlaw.

Genetic type 2 diabetes with diabetic foot complications :

The results of the frequency distribution of the G and C alleles of the Q488H gene, using the Hardy-Weinberg equilibrium law, showed different results between the sample of groups with type 2 diabetes with diabetic foot and standard samples, as the G allele recorded 35% in the sample of people with type 2 diabetes compared to the C allele. Which scored 65%, while the G allele scored 30% in the standard sample compared to C, which scored 70%, as shown in the figure (4-1). And the G allele appears significant compared with the C allele, which did not show any significant significance at the level of probability $p \leq 0.05$, and using the Fisher test, the critical ratio (OR) was 1.2564 with a confidence interval (CI) under 95% that ranged between 2.2736-0.6943, as shown in the table (4-1),(4-2) and showed The genotype homozygous for the GG alleles of diabetics with diabetic foot complications was highly significant (10245 0.0) with a probability level of $p \leq 0.05$ between the infected sample and the standard sample. From the standard sample using the Fisher test, the critical ratio (OR Odds ratio) was 00.3 with a confidence interval (CI) under 95%, a value that ranged between 11.4449 - 786.0 and the genotype showed heterogeneity in the CG genotype in patients with foot. Diabetes mellitus was highly significant compared to the standard sample, where the G allele showed a significant frequency in the infected sample with a rate of 0.024721, higher than the standard sample using the Fisher test, and the critical OR was 5.1 with a confidence interval (CI) under 95%, a value that ranged between (8661.3-582.0).

Here it must be noted that many factors, including environmental and genetic factors, are involved in the etiology of T2DM. Several studies have reported a role for genetic polymorphisms in the initiation and development of T2DM. In addition, most genome-wide association studies have identified more than 200 susceptibility loci, but it may be unclear whether these loci are associated with the pathophysiology of the disease. It has been indicated that possible genetic mechanisms underlying T2DM. It found that some genetic polymorphisms were associated with T2DM, either in the form of single-nucleotide polymorphisms or direct

amino acid changes in proteins. These gene polymorphisms are potential predictive markers of reduction in T2DM.(Panpan et al., 2017).

This study agrees with (Panpan, et al, 2017), where it was demonstrated by objectively evaluating the relationship between the Q488H polymorphism and T2DM through OR analysis and 95% CI under the genetic distribution. The result showed that the (Q488H) G allele was is a risk factor for T2DM and that subjects with the Q488H G allele have a higher vulnerability risk of developing T2DM compared to those without the G allele. Furthermore, interpretation of the variance between studies found in this field should be undertaken with caution; Clearly, heterogeneity exists in healthy volunteers and negatively selected populations. Other contributing factors may be the variable identification of T2DM patients and controls, genotype errors, and environmental history.

This study agrees with (Kanjana ; 2014) that the association between metabolic syndrome increases the risk of cardiovascular disease and type 2 diabetes. Adiponectin is a protein secreted by adipocytes with insulin-sensitive and anti-atherosclerotic properties. The identification of links between polymorphisms, (C>G) and (T>G) of the adiponectin gene, with respect to adiponectin levels and the metabolic syndrome, where the levels of adiponectin and HDL-C of the metabolic syndrome group were significantly lower than the control group ($P < 0.001$). Decreased adiponectin concentration was associated with a C>G polymorphism, This polymorphism was more common in the metabolic syndrome group than in the control group. However, a T>G polymorphism of the adiponectin gene was found to be unrelated to adiponectin level or to the metabolic syndrome. Therefore, the C>G polymorphism was associated with susceptibility to the metabolic syndrome, and this polymorphism influenced the distribution of adiponectin concentrations among Thais. This study agrees with (Yaofu Fan et al., 2015), where he showed that the G allele showed frequency and association with type 2 diabetes in some Asian human races. This study is consistent with (Monika et al., 2008) where it was shown that the association of the GG genotype and the G allele with DN, and all GG homozygotes in the group of patients had LDL cholesterol levels (low-density lipoprotein) higher than the homozygotes (AA) ($P < 0.01$), indicating that the effect may be related to the cardiovascular risk factor. These patients progressed faster to end stage renal failure than those with other genotypes.

It was pointed out by (Michael et al .,2019) that although non-genetic environmental factors play a major role in causing the development of type 2 diabetes, and because susceptibility to type 2 diabetes will differ between individuals, which makes the researchers say that the disease caused by inheritance. also. And genetics are mentioned to play an important role in the development of diabetes. Gene studies were the studies used to identify genes associated with type 2 diabetes before the advent of genome-wide association and genome-wide association studies, but only a few genes and variants were identified. It was indicated that linkage is a genetic tendency in which genetic markers are inherited together due to proximity to each other on the same chromosome. Genetic association analysis focuses on genomic regions with significant genetic influence that can influence disease progression. Genome-wide association assays have established that certain genes associated with type 2 diabetes are located on specific chromosomes. Association studies have been used to identify genes associated with various chronic disorders with unknown etiology such as type 2 diabetes, but many researchers consider them unsuccessful because they have identified only a few genes.

On the other hand, (Flora ,2012) referred to this case-control study to investigate the role of HSP90 (Gln488His (C>G)). The HSP90 GG genotype has been associated with a higher risk of breast cancer. Similarly, the allele dose-response model indicated an increased risk of breast cancer for each G. allele, as demonstrated by dose-response allele models. There appears to be a positive association between the HSP90 G allele and breast cancer risk. HSP90α Gln488 polymorphism appears to be a risk factor for breast cancer.

This study is consistent with what was reported by (Altaf .,2018) where genotyping revealed a significant association of the CG genotype with arrhythmia (cardiac physiology), indicating the presence of a heterozygous causative defect in patients. The GG and CG genotypes showed a significant association with this disease. Individuals harboring the heterozygous “CG” genotype are at increased risk of infection. In particular, females harboring the “CG” and “GG” genotypes can pass the risk allele to the future generation, Which leads to a heart disorder.

Table (4-1)eHSP90 Q488H polymorphism C/G POLY genotype frequency in patients with DM with DM foot and control group.

eHSP90 Q488H	Patients *n* = 50	Control *n* = 50	*P*	OR	95%CI
G	35	30	0.03502	1.2564	0.6943-2.2736
C	65	70		0.7959	0.4398-1.4403

n:number of alleles; OR: odds ratio; CI: confidence interval; HS: highly significant at P ≤ 0.05

Table (4-2): *eHSP90 Q488H polymorphism C/G* POLY genotype frequency in patients with DM with DM foot and control group.

eHSP90 Q488H polymorphism C/G	Patients *n* = 50	Control *n* =50	*P*1	*P*2	OR	95% CI
GG	15	10	0.010245 HS	0.040011 S	3.00	0.7864 - 11.4449
CG	5	10		0.024721 HS	1.5	0.582-3.8661
CC	30	30		Reference	Reference	Reference

HS: highly **n: number of alleles; OR: odds ratio; CI: confidence interval; S: significant at P ≤ 0.05 significant at P ≤ 0.05.**

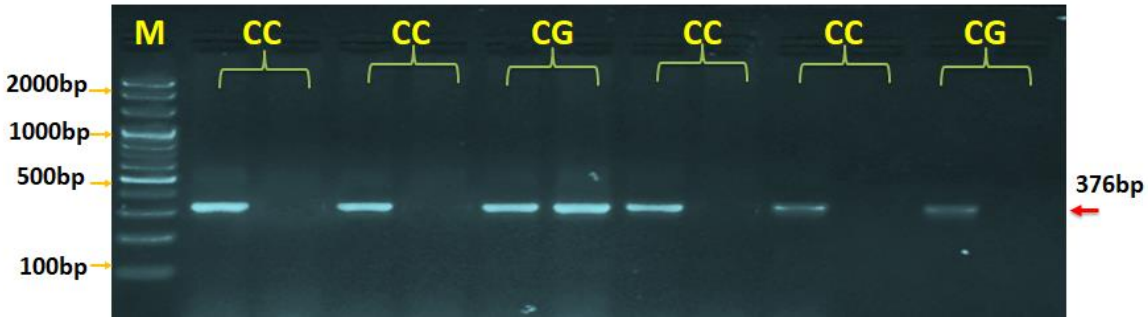

Figure (4-1): Agarose gel electrophoresis image that showed the ARMS-PCR product analysis of HSP 90 Q488H gene polymorphism in heathy control samples. Where M: marker (2000-100bp). The lane (CC) wild type homozygote were showed as C allele only. The lane (GG) mutant type homozygote were showed as G allele only, whereas the (CG) heterozygote were showed as both C and G allele. The presence of C or G allele were observed at 376bp product size.

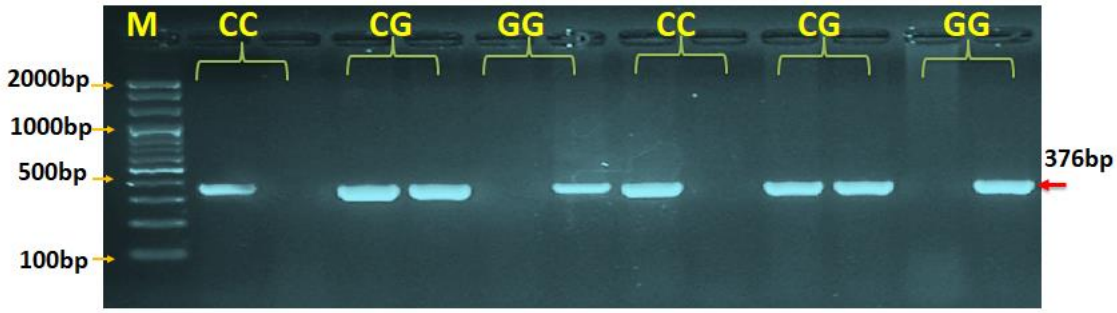

Figure (4-2): Agarose gel electrophoresis image that showed the ARMS-PCR product analysis of HSP 90 Q488H gene polymorphism incomplications of diabetic footpatients samples. Where M: marker (2000-100bp). The lane (CC) wild type homozygote were showed as C allele only. The lane (GG) mutant type homozygote were showed as G allele only, whereas the (CG) heterozygote were showed as both C and G allele. The presence of C or G allele were observed at 376bp product size

Conclusions and recommendations:

- Molecular study showed that there is an association between polymorphisms of a geneQ488H for heat shock proteins 90. Where a significant increase appeared in the allele G frequency in the infected samples compared with the control group, and the genotype GC appeared to be repeated with high significance.

Recommendations:

1 - Increasing the sample size to include multiple regions and different races.

2 - Adoption of this study to diagnose future complications for patients with type 2 diabetes.

3 - Adopting genetic diagnosis for the early detection of people with type 2 diabetes, taking into account the environmental aspect, balanced and healthy food, exercise, and the approved lifestyle.

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