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**INTERNATIONAL JOURNAL OF  
 ADVANCED RESEARCH (IJAR)**

Article DOI: 10.21474/IJAR01/7001  
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/7001>



### RESEARCH ARTICLE

#### DETECTION OF GUANINE NUCLEOTIDE BINDING SUBUNIT BETA B (GNB3) GENE IN SOME IRAQI PATIENTS TYPE2 DIABETES MELLITUS.

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#### Manuscript Info

##### Manuscript History

Received: 20 February 2018  
 Final Accepted: 22 March 2018  
 Published: April 2018

#### Abstract

Diabetes mellitus is one of the most common chronic diseases in nearly all countries, and continues to increase in numbers and significance. Various environmental and genetic factors interact and increase the risk of T2DM and its complications. G proteins are signal transducers that communicate signals from many hormones, neurotransmitters, chemokines and autocrine and paracrine factors. So it is represent some of the best examples of genetic influences that are involved in the determination T2DM.

characters.

**Methods:** The study include 37 T2DM without complication and 68 T2DM with complication patients carried out The Specialized Center For Endocrinology And Diabetes (AL-KindyHospital) in Baghdad-Iraq. For the purpose of comparison, 50 control subjects were matched for age, gender and ethnic background (Iraqi Arab).

**Results:** The result showed all samples (patients and controls) bands when analyzed by the wizard genomic DNA purification Kit (Intron, Korea), also the result revealed that the amplified DNA products band of GNB3 gene at level 268 bp in patients and controls.

**Conclusion:** our study revealed that the GNB3 could have an important role in the development of T2DM.

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#### Introduction:-

Type 2 diabetes mellitus (T2DM) is one of the most prevalent metabolic disorders worldwide, the prevalence of diabetes worldwide has increased dramatically and, in Iraq approximately 668,000 adults affected in year 2000 and this is expected to rise to 2 million by 2030 (ADA, 2016) (WHO, 2014). T2DM is a heterogeneous disease, resulting from the interaction of both genetic and environmental factors. It is not genetically determined, but the hereditary component of susceptibility to the disease is very strong. Several genetic loci are probably involved in this susceptibility (Dzida et al, 2002).

Guanine Nucleotide -Binding Proteins (G proteins) are a family of proteins involved in second messenger, that communicate signals from many hormones, neurotransmitters, chemokines, and autocrine and paracrine factors. Heterotrimeric G-proteins are made up of 3 subunits – alpha ( $\alpha$ ), beta ( $\beta$ ) and gamma ( $\gamma$ ). The beta ( $\beta$ ) subunit encoded by the GNB3 gene, in human. GNB3 gene that is present on chromosome 12 at the location of and

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is composed of 11 exons and 10 introns. The polymorphism(C825T) resulting from cytosine-to-thymine substitution at position 825 (Roskopf et al, 2000).The T825 variant of the gene is known to be associated with enhanced signal transduction via the G protein system (Andersen et al,2006).The activation of G-proteins stimulates adenylyl cyclase. This in turn induces hormone-sensitive lipase in adipose tissue, protein kinase A (PKA), and glycogen phosphorylase in muscle and fat cells, as well as in hepatocytes.Persistent stimulation may lead to insulin resistance and an increase in hepatic glucose output. G proteins also regulate $\beta$ phospholipase C (PLC  $\beta$ ), which produces phosphatidylinositol (IP3), the calcium channel activator. The opening of calcium channels initiates insulin secretion. Therefore, G proteins may contribute to the main path physiological mechanisms involved in type 2 diabetes, and the genes encoding its particular subunits areamong the candidate genes for this disorder (Chandrasekaran et al,2012).

### Material and Methods:-

#### Samples Collection:-

The control group consisted of 50 normal healthy subjects (25 males and 25 females) with mean age ( $56.4 \pm 9.2$  years). The patients group consisted of 105 T2DM patients (50 males and 55 females) with mean age ( $55.3 \pm 0.8$  years).

#### Genomic DNA Extraction:-

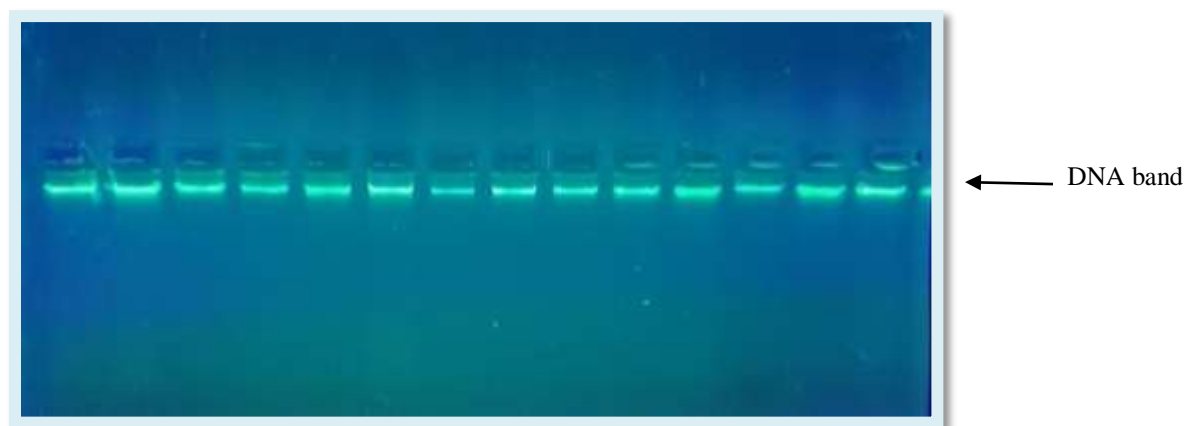
The DNA of the samples was extracted According to instructions (DNA purification kit, INTRON), DNA isolation from 200  $\mu$ l from the whole blood cells . The size of the extracted DNA was 30  $\mu$ l.The extraction was qualitatively confirmed using 1% agarose gel electrophoresis and quantitatively analyzedusing UV spectrophotometer (Scientific, USA).

#### PCR Amplification:-

The polymorphism was detected using PCR amplification using specific primers for GNB3 gene and their sequences were chosen according to Iyer et al,( 2014).Forward 5-TGACCCACTTGCCACC CGTGC-3. Reverse 5-GCAGCAGCCACCGCTGGC-3. The polymerase chain reaction (PCR) was performed in 25  $\mu$ l (Template DNA 1.5  $\mu$ l, Primer Forward 1 $\mu$ l F, Primer Reverses 1  $\mu$ l R, Deionized Water 16.9  $\mu$ l, PCR Master Mix 5  $\mu$ l). Thermal cycling conditions for the GNB3 were: initial denaturation at 94°C for 3 mint, followed by at 35 cycle program with denaturation at 94 °C for 30 S, annealing at 68 °C for 30 S, elongation at 72 °C for 30 S and final elongation at 72 °C for 7 mint.The PCR products were analyzed by electrophoresis on a 1.5% agarose gel stained in 0.5 mg/ml Red stain, product size of 268 (bp) have been patented.

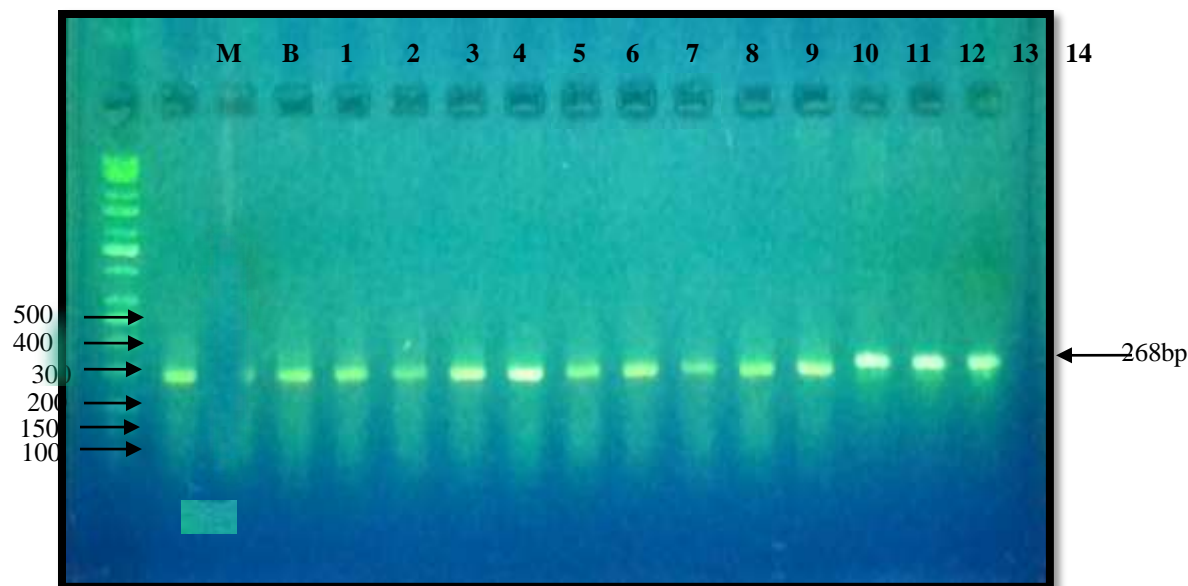
### Result and Discussion:-

The genomic DNA was isolated from whole blood cells using purification DNA kit (Intron, Korea) for patients and controls. The nucleic acid concentration and purity ration were automatic calculated by Nano Drop software and the results were as follows (1.8- 2) ng/  $\mu$ l.. All samples showed bands, which indicated the genomic DNA on the gel electrophoresis, figure (1).



**Figure (1):-** Agarose gel electrophoresis of the genomic DNA samples (total DNA). Fragments were fractionated by electrophoresis on 1% agarose (30min/70v), TBE 1X (tri-borate buffer) and visualized by safe red staining.

GNB3 gene was amplified in (155) samples (105 patients and 50 controls). Agarose gel electrophoresis of amplified DNA products showed the band of GNB3 gene at level 268pb. (Figure 2)



**Figure (2):-** A comprehensive PCR analysis of GNB3 polymorphism. GNB3 genes PCR product resolved by (2%) agarose gel electrophoresis (1hr/70v). Lane M, DNA molecular weight marker. Lane B, negative control. Lane (1-14) is samples.

### Conclusion:-

The current study revealed that the GNB3 gene fragment is located in 208bp.

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