

**Ministry of Higher Education &
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College of Education for Pure Science**



**Variations in Blood Adhesion Genes and Ulcer Foot
Bacterial Biofilm Genes: Effect of Copper Oxide
Nanoparticles on Bacterial Biofilm**

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Doctor of Philosophy in Biology

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1- Introduction

Diabetes is either inherited or acquired or both that make it as multi-gene diseases, and this applies to type 2 diabetes. Genetic susceptibility for diabetic with interaction of genetic and environmental factors create type 2 diabetes. The risk for developing the disease is 40% with one parent having type 2 diabetes, and 70% with both parents suffering from diabetes According to the American Diabetes Association 2016.

Studies have shown of 2115 non-diabetic patients followed for more than five years, the results showed that individuals with a family history of diabetes with FBG more than 5.5 with BMI over 30 had a 16-fold increased risk of type 2 diabetes. (Al-Hasnawi., 2017).

Cell adhesion molecules (CAMs) are located on the cell surface involved in binding with the extracellular matrix (ECM) or with other cells in the operation called cell adhesion (Khalili and Ahmad, 2015). Selectins (type I transmembrane proteins) are a family of mammalian vascular adhesion molecules involved in the tethering and deceleration of cells in lymphatic and bloodstream on endothelium capillary (Choudhary *et. al.* 2015). They play a great role in the passage of leukocytes into inflammation sites by mediating the first attachment and rolling of leukocytes on vascular endothelium before to integrin dependent extravasation and arrest (Natoni *et al.*, 2016). Selectins are related to DMT2, another local study showed E-selectin that plays a role in Iraqi patients with DMT2 (Al-mohaidi *et al.*, 2014; Al-Hasnawi *et. al.*, 2017). There are three types of selectin: P-selectin, E-selectin, and L-selectin (Wu *et. al.*, 2014). The genes for the selectins are located on chromosome number one (Kneuer *et.al.* 2006). which make patients more ready for the complication the disease.

Studies have indicated that different genetic variants (Single Nucleotide Polymorphisms, SNPs) of the inflammatory markers were correlated to different diseases including T2DM and others SNPs related to disease (Rodrigues *et al.*, 2016).

The cell walls of both Gram-positive and Gram-negative bacteria contain toxic components that are potent virulence factors and have central roles in the pathogenesis of bacterial septic shock, a frequently lethal condition (Silhavy *et al.* 2016). Considered adhesion of virulence factors for gram-positive and negative bacteria and the formation of biofilms of pathogens resistant bacteria to antibiotics. (Schroeder *et al.*, 2017). There is a strong correlation between the microbes and the replication of resistant bacteria (Munita and Arias, 2015). Increased association between Multiple drug resistance (MDR) bacteria and diabetic foot ulcer increases the risk of amputation (Yoga *et al.* 2006). Microbial resistance has dramatically increased during the last three decades, and spread around the world wide. Most Pathogenic bacteria have developed many ways to resist almost highly used antimicrobials. These pathogens can cause a wide range of superficial infections, and life-threatening infections in immunocompromised patients (Li and Webster, 2017).

There are therefore emergency needing to find new antimicrobial molecules, with an innovative chemical structure, (WHO, 2015) like Nanoparticles which they have great antimicrobial properties due to their three dimension in surface size to large size, (Yoga *et al.* 2006). Copper is one of the nanoparticles used in modern experiments: that depend on its easy oxidation, selectively processes dual DNA, allowing for mass volume control (Tauran *et al.* 2013). Copper surfaces or alloys can eliminate 99.9% of pathogenic bacteria in hours, including methicillin-resistant

Summary

This study conducted on 100 Iraqi individual for 50 patients (30 males and 20 female), aged range between (43- 94) years that were diagnosed with Type 2 Diabetes Mellitus (T2DM) and foot ulcers, and 50 Healthy subject (27males and 23 female). Their ages ranged (27-66), who were periodic attended to the hospital of Baghdad and Baqubah, from February to December 2018. Questionnaire list (Appendix1) was prepared for T2 DM patients, and the controls who were characterized in terms of age, gender, family history of diabetes, duration of disease for patients, height (abdominal circumference), were which measured and recorded in cm, and body weight which was measured and recorded in Kg. The patients and controls were also determined for fasting blood glucose (FBG), glycated hemoglobin (HbA1c), lipid profile (TC, TG, HDL, LDL and VLDL), insulin, resistance insulin (HOMO IR), and Atherogenic index. In addition, the studies polymorphism for the adhesion gene and bacteria was done in the molecular biology laboratory in biology department College of Education for pure Science in the University of Diyala.

The aim of this study is to evaluate the relationship between Adhesion genes of diabetic foot ulcer and biofilm genes of bacteria, and on the other hand, the effect of copper oxide nanoparticle on isolated bacteria from foot diabetic ulcers.

The genetic polymorphism of L-selectin gene rs2205849 of the genotype AA showed high frequency ratio in patient group compared with control 67.5 and 56%, respectively with etiological risk factor 1.63 but non significant according to fisher's probability.

The results of the E-selectin gene polymorphism showed five SNPs in the E-Selectin Leu554Phe gene amplification region. The amplified portion that was extended from the end of intron 8 to exon 11 of the E-Selectin gene contains the

Staphylococcus aureus, *Escherichia coli*, *P. aeruginosa*, *Monoclonal Listeria*, *intestinal Salmonella*, *Camylopactropylica*. (Sánchez, 2016).

1-2 Aims of the study:

- 1- Study SNPs in human selectin L and E genes and sequencing of some segments of selectin genes.
- 2- To Evaluation the relationship between Adhesion genes of Diabetic Foot Ulcer patients and bacterial adhesion genes
- 3- To Evaluation the effect of nanoparticle on commonly isolated bacteria from Foot Diabetic Ulcers.