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Association between pro-inflammatory cytokines and some viral infections in Beta-Thalassemia patients

Saja Mohammed Mohsen
Middle Technical University, Baquba -Technical College, Diyala, Iraq

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ABSTRACT

Thalassemia is a hereditary hemoglobin production defect. Thalassemia major (TM) individuals need periodic blood transfusions to maintain an appropriate level of hemoglobin for supplying oxygen systems. Because of transfusion, these individuals have various problems such as infections, autoimmune diseases, and alloimmunization. The current research focuses on detecting the predictive roles of IgG, IgM, IL-8 and TNF-α in the pathogenesis of beta-thalassemia disease. The research was conducted in the Baquba/Diyala Governorate in Iraq from July to August- 2023. Fifty blood sample were obtained from beta-thalassemia patients (30 males and 20 females with age range among groups (1-30 years). In addition, fourteen samples were collected from Apparently individuals as a control group. IgG and IgM levels were detected by Cobas integra-400 plus machines. ELISA was used to determine the serum levels of the IL-8 and TNF-α. Most beta-thalassemia patients have no viral infections (66%), but a few of them have positive viral infections as follows: Hepatitis C virus (HCV) (8%), Hepatitis B virus (HBV) (14%), Parvovirus (B19) (6%), and HCV with HBV (6%). The levels of IgG and IgM and cytokines (TNF-α and IL-8) were significantly greater ($p<0.05$) in patients than in healthy ones. Most patients with beta-thalassemia are males less than 20 years old. A few of the patients have positive viral infections (HCV, HBV, and B19). The indicators of IgG, IgM, TNF-α, and IL-8 have significant role in the pathogenesis of beta-thalassemia due to their high sensitivity and specificity.

Corresponding Author:

Saja Mohammed Mohsen
Middle Technical University(MTU), Baquba Technical college
Baqubah City, Diyala Governorate, Iraq
Email: sajamohammad@mtu.edu.iq

1. INTRODUCTION

Thalassemia is a multigenetic inherited illness that includes alpha, beta, delta and beta thalassemia, among others. It is a genetic ailment that implies a minimum of a single parent must be a carrier of the illness [1]. It occurs by either a genetic abnormality or a missing portion of specific significant regions of the genes. Molecular abnormalities occur in incorrect hemoglobin synthesis in a group of the beta-globin gene on the eleventh chromosome and the sixteen chromosomes located in the alpha-globin gene group [2]. Thalassemia disorders with numerous clinical manifestations, characteristics, and treatment choices are classified on an intensity scale. Thalassemia is classified into two categories: TDT (transfusion-dependent thalassemia) and NTDT (nontransfusion-dependent thalassemia) [3]. Excess iron has been linked to substantial morbidity (damage to numerous essential organs) in both transfusion-dependent and nontransfusion-dependent thalassemia patients [4]. Beta-thalassemia affects approximately 1.5% of the global population (80-90 million persons), with approximately sixty thousand individuals who are newborns with abnormalities worldwide, the vast majority of whom from nations with limited development [5]. According to worldwide estimates, the average yearly occurrence of symptomatic patients is one in 100,000. With an inheritance rate of approximately 50% [6], beta-thalassemia with irregular Hb or structural Hb types with thalassemia features has become the most prevalent combination in this region of southeast Asia. In the nation of Iraq, thalassemia accounts for 75% of all hemoglobinopathies. The Basra governorate has also a greater incidence of thalassemia, which accounts for 67% of all types of thalassemia [7].

Blood bone illnesses are the 2nd leading cause of mortality in thalassemia individuals with thalassemia. Repeated blood donations expose thalassemia patients to an increased risk of transmissible viruses (HBV, HCV, HAV, and parvovirus B19), especially if sufficient viral testing of those who donate blood is not performed [8]. In thalassemia patients , a wide range of immunological abnormalities has been found [9]. These quantifiable and functional deviations affect a variety of immune-related elements. In this category, a changed cytokine profile related to natural immunity, in addition to the existence of a low-grade systemic inflammation state represented by higher total leukocyte, neutrophil, and lymphocyte counts, has been shown [10]. The investigators discovered that those suffering from thalassemia had significantly greater levels of IL-8, IL-13, and TGF-β than did normal controls [11].

IL-8 is a potent proinflammatory cytokine that promotes neutrophil migration through the capillary wall (chemotaxis). This molecule is a member of the interleukin superfamily, which comprises neutrophil-activating peptide-2, platelet factor-4, growth-related cytokine (GRO), and interferon-inducible protein-10; all of these molecules are involved in the movement of cells [12]. The investigators discovered higher levels of IL-8 in individuals with thalassemia than in controls [9]. A further investigation was made by Hanoon and Al-Mudalal (2018) who revealed that individuals with thalassemia who underwent splenectomy had higher levels of IL-8 than nonsplenectomized and control individuals [13]. Tumor necrosis factor-alpha TNF-α is a mediator with pleiotropic impacts on a variety of cells. It was identified as an essential regulator of inflammatory reactions and is thought to play a role in the development of various inflammatory conditions and autoimmune illnesses [14]. Immunoglobulins Igs or antibodies are glycoproteins generated by B lymphocytes when they are exposed to immunogens (microorganisms, cellular antigens, chemicals, and synthetic compounds) [15]. There are a variety forms of immunoglobulins, including IgG, IgM, IgD, IgA, and IgE [16]. There are several immunological problems related to beta thalassemia, including impaired opsonization and cellular phagocytosis, higher levels of serum immunoglobulins (IgG and IgM), and excess iron, all of which harm the body's defenses [17]. Due to the defective immune status of patients with beta thalassemia, the present research intends to investigate the predictive it mmunoglobulins (IgG and IgM) and cytokines (IL-8 and TNF-α) in beta thalassemia disease.

2- METHODS

2.1 Sampling collection

The current research was conducted in the Baquba/Diyala Governorate in Iraq for the period between July to August 2023. Fifty blood sample were obtained from beta-thalassemia patients (30 males and 20 females age range among groups of (1-30 years). The patients were admitted to the Blood Diseases Center/Baquba Teaching Hospitals after being diagnosed by a hematologist-physician. In addition, fourteen samples were collect from parentally individuals and as a control group. A questionnaire with information about age, sex, and splenectomy status was completed by the patients and healthy controls.

2.2 Immunological test

Three ml of blood collected from patients were centrifuged 5000 rpm for five minutes to obtain serum that preserved in -20 °C. The IgG and IgM levels were detected by Cobas integra-400 plus machines. The serum levels of the IL-8 and TNF-α were measured via enzyme-linked immunosorbent assay (ELISA) according to the manufacturers procedures (CAUSABIO, China).

2.3 Statistical analysis

The IgG, IgM, IL-8 and TNF-α levels were presented as the means±SDs, and Student's t tests were used to determine the significance of the differences (patients versus healthy individuals) or F tests (splenectomized patients vs. nonsplenectomized patients vs. healthy individuals). The data for the current research were analyzed using SPSS version 25.0 and GraphPad Prism version 6 statistical software. Demographic features (age and gender), as well as splenectomy, are presented as frequencies and percentages, and the Pearson-Chi-square test detected differences among percentages. Receiver operating characteristic (ROC) curves were used to determine the area under the curve (AUC), cutoff, specificity, and sensitivity of the levels of immunoglobulin IgG and IgM and the cytokines IL-8 and TNF-α. $P \leq 0.05$ was considered to indicate a significant difference.

3-Results

3.1 Demographic features and splenectomy operation of beta-thalassemia patients

The current research revealed that the percentages of beta-thalassemia patients are greater in the males (60 %), aged ≤10 (32%), 11-20 years (40%) and nonsplenectomized groups (76%) than in the females (40%), aged 21-30 years (18%), 31-40 years (10%), and splenectomized groups (24%), with significant differences ($P<0.05$).

3.2 Relationship between viral infections and beta-thalassemia patients

Based on the obtained results, most beta-thalassemia patients have no viral infections (66%), but a few of them have positive viral infections as follows: HCV, HBV, B19. The variations in the positivity of viral infections are significant ($p<0.05$) as presented in Figure 1.

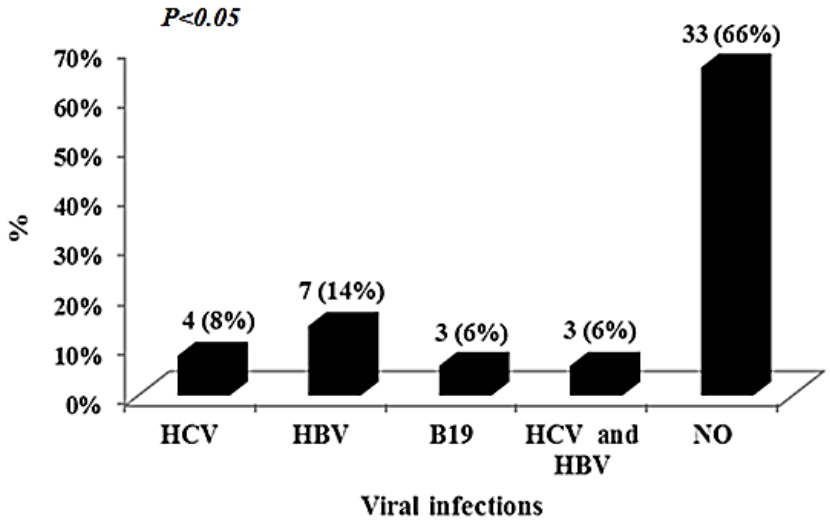

Figure 1. Distribution of the HCV, HBV and B19 in beta-thalassemia patients

3.3 distribution of immunoglobulins (IgG and IgM) and cytokines (TNF-α and IL-8) in the study groups

The current research revealed greater levels of immunoglobulins (IgG and IgM) and cytokines (TNF-a and IL-8) in patients (19.39±6.32, 3.81±1.2, 32.71±12.71, and 68.51±20.86) than in healthy individuals (12.21±4.08, 1.52±0.62, 22.87±7.31, and 51.81±18.24, respectively) with ($p<0.01$) as depicted in Table 1.

Table 1. Comparison of the mean levels of immunoglobulins (IgG and IgM) and cytokines (TNF-α and IL-8) between patients and healthy controls

Parameters	Patients (n=50)		Healthy (n=30)		P value
	Mean	SD	Mean	SD	
IgG (g/L)	19.39	6.32	12.21	4.08	P <0.01*
IgM (g/L)	3.81	1.2	1.52	0.62	P <0.01*
TNF-α (pg/ml)	32.71	12.71	22.87	7.31	P <0.01**
IL-8 (pg/ml)	68.51	20.86	51.81	18.24	P <0.01**

3.4 Comparative mean levels of (IgG, IgM, TNF-α, and IL-8) with splenectomized and non splenectomized in beta-thalassemia patients

The present research indicated greater levels of IgG, IgM, TNF-α and IL-8 in splenectomized patients (21.82 ±8.32, 4.84 ±1.47, 36.14 ±14.78, and 75.89 ±25.71, respectively), and the non splenectomized patients of (12.21±4.08, 1.52±0.62, 22.87±7.31, and 51.81±18.24, respectively), as elaborated in as Table 2.

Table 2. Comparative mean levels of immunoglobulins (IgG and IgM) and cytokines (TNF-α and IL-8) among the study groups

Parameters	Splenectomy (n=12)		Non-Splenectomy(n=38)		Healthy (n=30)		P value
	Mean	SD	Mean	SD	Mean	SD	
IgG (g/L)	21.82 a	8.32	16.51 b	4.41	12.21 c	4.08	P <0.01**
IgM (g/L)	4.84 a	1.47	2.71 b	0.95	1.52 c	0.62	P <0.01**
TNF-α (pg/ml)	36.14 a	14.7	28.85 b	10.03	22.87 c	7.31	P <0.01**
IL-8 (pg/ml)	75.89 a	25.7	63.62 b	16.06	51.81 c	16.2	P <0.01**

Lowercase letters indicate significant differences ($P<0.05$).

3.5 Receiver operating characteristic (ROC) curves of immunoglobulins (IgG and IgM) and cytokines (TNF-α and IL-8)

The immunoglobulins IgG and IgM showed the highest sensitivity and specificity at the cutoff, followed by TNF-a and IL-8, which have the lowest sensitivity and specificity at the cutoff, for screening beta-thalassemia patients. Significant differences of ($p<0.01$) are shown in Table 3.

Table 3. ROC curve, cutoff, sensitivity and specificity of immunoglobulins (IgG and IgM) and cytokines (TNF-a and IL-8)

Parameters	AUC*	St. error	P value	Cutoff	Sensitivity %	Specificity %
IgG (g/L)	0.950	0.045	P<0.01**	14.00	100	80
IgM (g/L)	0.880	0.076	P<0.01**	1.65	90	72
TNF-α (pg/ml)	0.810	0.101	P<0.05*	24.50	84	71
IL-8 (pg/ml)	0.840	0.093	P<0.01**	60.50	88	75

*AUC= Area under the curve. Different letters indicate significant differences (P<0.05)

4. Discussion

As a hereditary condition, thalassemia is defined by inefficient erythropoiesis, which results in prolonged anemia and impaired iron metabolism [18]. These individuals require monthly blood transfusion to avoid consequences such as persistent anemia and bone alterations, which cause iron excess in their systems. Chelation treatment has advanced in the past few decades to reduce excess iron in the body, although endocrinopathies continue to endanger people's quality of life [19]. The incidence of thalassemia increased in individuals aged 10 years (32%) and 11-20 years (40%), which contradicts the findings of Gharehdaghi et al. (2023), who reported an increased incidence of thalassemia in individuals aged between 21-30 years (39.0%) and 31-40 years (34.0%). Gharehdaghi et al. (2023) also reported a greater incidence of thalassemia in males than in females ($p<0.05$), and these findings are consistent with the current findings [19]. Albahout et al. (2023) [20] reported that the majority of people diagnosed with thalassemia are between 1 and 30 years old (67.0%), which is consistent with the results of this research. Another investigation revealed that those in the most susceptible group of people to become sick are between 11-19 years old, and it appears that this proportion decreases with age, as do the proportions and data of older individuals. This suggested that blood transfusions and chelation therapy can expand the lives of these individuals. The disparity might be attributed to sample size.

Banafa et al. (2022) indicated that men (65.54%) outnumbered girls (34.46%) and this difference was significant ($p=0.05$) [21]. These findings are consistent with the current findings. In contrast, Rambod et al. (2023) reported that females (72.54%) had a greater prevalence of thalassemia than men (27.46%). This discrepancy might be attributed to differences in heredity and population [22]. Hepatitis C virus (HCV) infection is a problematic transfusion-transmitted illness in the community, with more than forty percent fatality in those with thalassemia [23]. The best strategy to manage this illness in the event of a lack of preventive vaccination is to enhance blood donation security. The incidence of HCV in individuals with thalassemia can be utilized to assess the safety of blood screening in various countries [24]. Sadulla et al. (2020) [25] elucidated a high prevalence of the HCV virus (35%) (diagnosed by ELISA) in Beta-Thalassemia patients in Northern Iraq, and these results were greater than the findings of the results in Diyala Province, which showed the lowest percentage (8%). In another study in Pakistan, the authors showed that 37% of beta-thalassemia patients are positive for HCV [26]. In Iran, the authors reported that 17% of beta-thalassemia patients are positive for HCV [23]. In Al-Diwaniya Province, Jallab and Easa (2020) [27] stated that 2.5% of beta-thalassemia patients are positive for HBV, and these results are lower than the current results of Diyala Province, which showed that 14% of patients are positive for HBV. In another study in Kirkuk Province, Qassim Welli et al. (2021) elucidated that (<2%) of patients are positive for HBV [28]. A study in Thi-Qar Province, Iraq, Alnassar and Shallal (2023) [29] showed that 11% of people with beta-thalassemia had the cheerful Parvovirus B19, and these findings are the highest ones compared to the findings in Diyala Province, Iraq, in which 6% of patients have the cheerful Parvovirus B19. Another study was conducted by Soltani et al. (2020) who showed that 21% of people with beta-thalassemia are infected with the sensitive Parvovirus B19 [30]. In contrast, Sadah and Al-marsome (2020) [31] conveyed that 9% of beta-thalassemia patients with severe Parvovirus B19 (diagnosed by PCR were diagnosed with this virus, and these results are the lowest compared to the above results). The differences among these studies are related to the diagnostic technique used. The differences among these studies regarding the positivity of HCV, HBV, and parvovirus B19 in patients with thalassemia are related to sample size, diagnosis methods, vaccination, treatment, and control conditions of blood transfusion.

Although blood institutions have regular protocols for ensuring blood protection, the use of HBV, HCV, and Parvovirus B19 provides substantial difficulty in the care of those suffering from thalassemia. There is still a significant chance of developing a viral infection. On the other hand, people with thalassemia are at a low risk of HBV infection. Providing HBV vaccination in combination with HCV therapy with direct-acting antivirals (DAAS), as well as sufficient iron chelation, assuring an immunological state, and tracking hepatitis indicators, may significantly reduce the prevalence of viral hepatitis among patients [32]. Ehsanipouret et al. (2022) [33] described that patients who died had significantly greater quantities of IgG and IgM than healthy subjects/ This agrees with the current findings. An increase in the serum immunoglobulin concentration is caused by ongoing exposure to different antigens, and new infections may also boost the immune system's response, resulting in elevated immunoglobulin levels. Excess iron has been proposed to be a significant risk factor influencing immune system indicators in patients with thalassemia disorders [34].

In the present research, the levels of IgG and IgM in individuals are increased, which may be attributed to improved chelation and leuco-depleted red blood cell transfusions, which are currently used for thalassemia patients. The amount of immunoglobulins increased as the amount of transfusions increased [34]. It was recently proposed that donor alloantigens induce the generation of alloantibodies against patient-derived RBCs, causing conformational modifications on the receptor of the antigen on the RBC and, therefore, driving the generation of autoantibodies. Furthermore, leukocyte proteins are a significant source of immunologically activated proteins. Leuco depletion, ideally before preservation, is consequently required to diminish all autoimmunization in those with thalassemia [35]. Hanoon and Al-Mudalal (2018) [13] reported that patients who are thalassemia have significantly greater amounts of IL-8 than healthy subjects. Again, this is in an agreement with the current findings. This increase might be attributed to the continual transfusion-related stimulation of antigens and excess iron, which leads to enhanced IL-8 generation among Thalassaemia individuals [36]. In these individuals, ailments, immunological deviations, hypercoagulability, and a greater likelihood of clotting are thought to be the leading causes of morbidity and death, with pathogenic problems perhaps the outcome of immune system dysfunction [36]. A previous study suggested that an irregular cytokine profile (more significant IL-8, IL-13, TGF-α, and reduced 9 L-4) can lead to iron overload and decreased immune cell functioning, indicating that dysregulated cytokines are suitable for the identification and assessment in future studies [11]. Caprari et al. (2023) [36] indicated lower levels of IL-8 in individuals with thalassemia who frequently received blood transfusions than in healthy controls. Nevertheless, these findings are not consistent with the current findings. The decrease in IL-8 concentrations is due to the harmful impact on immune system cells caused by excess iron generated by blood donations and inefficient erythropoiesis, which leads to an increased intake of iron from the stomach. Although iron chelator treatment can alleviate extreme excess iron, people who have thalassemia have elevated amounts of iron in several organs, including the liver, heart, and pancreas [36].

Previous studies revealed that blood transfusions could have an acute negative effect on lung activity in thalassemia patients, with disorganized pulmonary performance associated with elevated levels of IL-8 and TGF-β, suggesting that the immune system's response works to prevent the suppression of pulmonary function [11]. Recent research has suggested that cytokines may be implicated in poor erythropoiesis (EPO) in thalassemia patients. Previous studies have shown that inflammatory substances, such as TNF-α and interferon-γ, can produce nitric oxide, which is implicated in the death of progenitor cells of erythroids from thalassemia/Hb E individuals [37]. However, the process through which TNF-α is involved in EPO control is unknown. TNF-α is a proinflammatory cytokine that has been shown to suppress glycophorin A+ cell production, and diminished differentiated erythroid cells worsen inefficient erythropoiesis in beta-thalassemia patients [38]. Nasser and Maleek (2023) [39] reported higher concentrations of TNF-α in individuals than in healthy controls, which is consistent with the current findings. This might be attributed to iron excess and antigenic incentives, both of which are promoted by prolonged transfusion treatment. It was suggested that the increase in TNF-α was mediated by the activation of macrophages from excess iron and the stimulation of antigens from prolonged transfusion treatment. Activated macrophages phagocytose apoptotic erythroid precursors, resulting in inefficient erythropoiesis [39].

Previous research revealed that approximately 50% of individuals with beta thalassemia have elevated blood TNF-α levels and that alterations following bone marrow transplantation (BMT) are associated with the incidence of immune-mediated problems. Low TNF-a levels may persist after effective integration because of the use of the preparative regimen and the absence of unfavorable immunological responses. Researchers have found that patients with thalassemia have significant immune cell numbers but poor immune system function, probably as a result of antigenic exposure following blood transfusions, excessive iron intake, and splenectomy [40]. Splenectomy significantly reduced the initial immunoinflammatory reaction to multiple injuries and subsequent sepsis by inhibiting the presentation of antigens, the ejection of particular proinflammatory cytokines, and granulocyte stimulation without affecting phagocytic capability [40].

Because of the impaired immunological functioning of the body's immune system, splenectomies for thalassemia major have been demonstrated to be more effective. Hanoon and Al-Mudalal (2018) [13] found that the most significant amount of IL-8 in splenectomized individuals, followed by non splenectomized individuals, in contrast to normal controls, and these findings agreed with our findings. This could be attributed to an improvement and ongoing improvement in both monocytes and lymph cells, which are the sources of IL-8 following splenectomy [40].

The investigators found that IL-8 levels were significantly greater in the splenectomized group than in the nonsplenectomized and control groups, as those in the nonsplenectomized group compared to those in the control group. In addition, they found that spleen removal was hazardous to thalassemic individuals, particularly on an immunological basis [13]. Splenectomy has been shown in laboratory and clinical investigations to enhance TNF-α and cell death in a variety of illnesses [11]. Serum TNF-α levels were shown to be elevated in thalassemia patients, especially after spleen removal. TNF-α is a marker of monocyte stimulation, and serum levels of C-reactive protein were shown to be increased in sickle cell disease patients with functioning asplenia [40]. Sale et al. (2020) [20] ascertained no significant differences ($p>0.05$) in TNF-α levels among nonsplenectomized, splenectomized, and control group individuals, which contradicted the current findings. Sale et al. (2020) [20] demonstrated that splenectomized individuals had the most significant concentrations of TNF-α, followed by nonsplenectomized individuals and healthy controls. Another study revealed no significant differences in TNF-a levels ($p>0.05$) between nonsplenectomized, splenectomized, and control individuals [10]. The discrepancies across trials were linked to blood transfusion frequency, immunological state, and therapeutic medications.

The outcomes of the current investigation demonstrated that immunoglobulins (IgG and IgM) and cytokines that promote inflammation (TNF-a and IL-8) had high sensitivity and specificity in evaluating individuals with thalassemia because of their important involvement in the pathogenesis of this illness.

5. Conclusions

The current research has been concluded that most patients with beta-thalassemia are males of less than 20 years old. A few of the patients have positive viral infections (HCV, HBV, and B19). The indicators of IgG, IgM, TNF-α, and IL-8 have significant role in the pathogenesis of beta-thalassemia due to their high sensitivity and specificity. The levels of immunoglobulins (IgG and IgM) and cytokines (TNF-a and IL-8) in patients (19.39±6.32, 3.81±1.2, 32.71±12.71, and 68.51±20.86) than in healthy individuals (12.21±4.08, 1.52±0.62, 22.87±7.31, and 51.81±18.24, respectively) with ($p<0.01$) . Also The immunoglobulins IgG and IgM showed the highest sensitivity and specificity at the cutoff, followed by TNF-a and IL-8, which have the lowest sensitivity and specificity at the cutoff, for screening beta-thalassemia patients. Significant differences of ($p<0.01$) .

Ethics approval and consent to participate

The current research was approved by the Ethical Committee of Blood Diseases Center/Baquba Teaching Hospitals and Middle Technical University (MTU), Baquba -Technical college, Diyala, Iraq. Written consent was obtained from this center prior accordance with the code of Ethics in Hospitals in Iraq.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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BIOGRAPHIES OF AUTHORS :-

Dr. Saja M. Mohsen: is lecturer at Baquba Technical College , middle technical university. She Holds a PhD degree in Biology Science with specialization in microbiology . His research areas are virology , immunology , bacteriology and Genetics. She has published several scientific papers in national, international conferences and journals. She can be contacted at email: sajamohammad@mtu.edu.iq