

Full Length Research Paper

Prevalence of bacteremia in patients with diabetes mellitus in Karbala, Iraq

Mohammed A. K. Al-Saadi¹, Alaa H. Al-Charrakh^{1*} and Salim H. H. Al-Greti²

¹Department of Microbiology, College of Medicine, Babylon University, Babylon Province, Iraq.

²Medical Institute of Karbala, Karbala Province, Iraq.

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The present study is designed to study bacteremia and to measure some immunological parameters of diabetic patients in Kerbala City, Iraq during the period from November 2006 until May 2007. This study included a total of 125 patients with diabetes mellitus (30 type I and 95 type II), and 55 healthy persons as Control subjects. Blood samples were collected from both patients and Controls, blood culture was done for bacterial isolation and identification, virulence factors as well as antibiotic susceptibility tests were assessed for each isolate. This study also included the estimation of T-cells count, interferon-gamma (IFN- γ) concentration, interleukin-4 concentration, IgG, and IgM concentration. The obtained results showed that bacteremia was observed in 24% of the diabetic patients. Gram-positive bacterial isolates were more predominant; 21:30 (70%); than Gram-negative isolates; 9:30 (30%). Cefotaxime, tetracycline and trimethoprim-sulphamethazole antibiotics were the most effective drugs on both Gram-positive and -negative bacteria. Immunological tests showed decrease in T-cells count significantly ($p < 0.05$) in type I and II diabetic patients (9.1%, 10.63%, respectively). Concentration of IFN- γ also decreased significantly ($p < 0.05$) in same patients (0.285 and 0.313 I.U/ml, respectively) as compared with control subjects (0.860 I.U/ml). Levels of IL-4 decreased non-significantly ($p > 0.05$) in patients with type I and II diabetes (7.050 and 7.703 pg/ml, respectively). The levels of IgG were increased significantly ($p < 0.05$) in both types I and II (1674.45 and 2095.86 mg/dL) respectively, but the levels of IgM were increased significantly ($p < 0.05$) in type II (177.64 mg/dL) and non-significantly ($p > 0.05$) in type I.

Key words: Bacteremia, diabetes mellitus, antibiotics, IL-4, IFN- γ , Iraq.

INTRODUCTION

Diabetes mellitus (D.M.) is defined as an abnormal metabolic state in which there is glucose intolerance due to inadequate insulin action as well as the late development of many complications (Boon et al., 2006). The World Health Organization recognizes two main forms of D.M: Type I and II. Type I is usually due to autoimmune destruction of the pancreatic beta cells (β -cells) which produce insulin (WHO, 1999). Type II is a heterogeneous group of disorders characterized by tissue-wide insulin resistance and varies widely (Kasper, 2005). Diabetic subjects probably have a higher risk of

many infections. In addition, immune-suppression which occurs in those patients because of increased sugar levels in the blood stream and as a result of dysfunction of the immune system, make diabetic patients more prone to microbial infections, especially bacteremia (Joshi et al., 1999). Several factors could predispose diabetic patients to infections. These factors include genetic susceptibility to infection; altered cellular and humoral immune defense mechanisms; local factors include poor blood supply and nerve damage and alterations in metabolism associated with diabetes (James, 2000). Chronically ill and immunocompromized patients with D.M have an increased risk of bacteremia. They may also develop bacteremia and fungemia (Vincent et al., 2004). On the other hand, their antimicrobial function is inhibited by hyperglycemia due to inhibition of

*Corresponding author. E-mail: aalcharrakh@yahoo.com. Tel: 009647707247994, 009647813216822.

glucose -6- phosphate dehydrogenase (G6PD) (Thomson et al., 2004). Another mechanism which can lead to increased prevalence of infections in diabetic patients is an increased adherence of microorganisms and they grow better in glucose, in addition to their expression of different virulence factors (Geerlings and Hoepelman, 1999). Impaired host defense mechanisms such as impaired granulocyte function, decreased cellular immunity, impaired complement function and decreased lymphokine response may be influenced by glycemic control (James, 2000).

The objective of this study was to investigate the etiologic agents of bacteremia in patients with diabetes mellitus and study the virulence factors and antibiotic sensitivity pattern among recovered bacterial isolates, in addition to studying some humoral and cellular immunological parameters in diabetic patients with and without bacteremia. Also a comparison was made between diabetic patients and the healthy control group.

MATERIALS AND METHODS

Patients and samples collection

This study included a total of 125 diabetic patients as diagnosed by clinical physicians (35 Type I and 90 Type II) with age ranging between 17 to 65 years who attended Al-Hussein Hospital in Kerbala, Iraq during the period from November 2006 until May 2007. Case history of patients involving patient's name, age, type of therapy and accompanying disease were recorded. Only diabetic patients with no malignancy, asthma, cardiovascular accident, heart failure and with no previous administration of antibiotics were considered in the present study. Also, a total of 55 healthy persons (volunteers) with age matching to the patients group were enrolled in the present study as control subjects.

Blood samples (withdrawn by disposable syringe under aseptic techniques) were collected from each patient and control subject. The blood samples were divided into three parts; 1 ml was immediately inoculated into sterilized blood culture bottles for bacteriological investigation. 2 ml were put in EDTA anti-coagulant tubes for testing with the E-rosette technique. The remaining 3 ml were put in sterilized plane tubes and allowed to clot and then the serum was separated (by centrifugation at 3000 rpm for 15 min) and stored (refrigerated) until use for further immunological tests.

For bacteriological investigation, the blood samples were introduced directly into Brain Heart Infusion (BHI) broth bottles with the ratio 1:10 of blood to medium used. Blood culture bottles were incubated at 35°C for 7 days and shaken for the first 48 h. Bottles were tested for bacterial growth once on the day of receipt, twice on day 2, and once daily on day 3 through 7 (Forbes et al., 2007). Blood culture suspected of being positive were sub-cultured onto MacConkey agar (Mast, U.K.) plates of 5% human blood agar, and chocolate agar (supplemented with 10% CO₂). All agar plates were incubated for 48 h at 35°C.

Bacterial isolates

The isolation and identification of bacteria recovered from patient's blood culture were performed through colony morphology of bacterial isolates, microscopic Gram stain investigation and biochemical tests (Forbes et al., 2007). Isolates identification was also confirmed using API system strips (Biomérieux/France).

Detection of virulence factors

Virulence factors of bacterial isolates were detected according to standard procedures. The following virulence factors; coagulase, capsule production, lipase, gelatinase, hemolysin and colonization factor antigens; were detected according to Forbes et al., (2007). Bacteriocin production was assayed using cup assay method in Brain Heart infusion medium supplemented with 5% glycerol according to (Al-Qassab and Al-Khafaji, 1992).

Antimicrobial susceptibility testing

The susceptibility of the bacterial isolates to antimicrobial agents was determined using disk diffusion method as recommended and interpreted accordingly by the Clinical and Laboratory Standards Institute (CLSI, 2006; 2007). The following antimicrobial agents were obtained (from Oxoid, U.K.) as standard reference disks for known potency for laboratory use: Amoxicillin (Ax, 10 µg), cefotaxime (CTX, 30 µg), oxacillin (OX, 1 µg), cefoxitin (Fox, 30 µg), amikacin (Ak, 30 µg), tetracycline (Te, 30 µg), doxycycline (Do, 30 µg), ciprofloxacin (CIP, 5 µg), nalidixic acid (NA, 30 µg) and trimethoprim-sulphamethazole (SXT, 1.25/23.75 µg).

All tests were performed on plates of Muller- Hinton agar (Oxoid, U.K). A 0.5 MacFarland suspension (provided by Biomérieux/ France) of tested bacterial isolates was applied to the plates which were dried in an incubator at 35°C for 15 min. Antimicrobial disks were placed on the agar with sterile forceps. The agar plates were incubated and inverted at 35°C for 18 h. Results were recorded by measuring the inhibition zone (mm) and interpreted according to Clinical and Laboratory Standards Institute documents (CLSI, 2007).

Estimation of immunological parameters

Erythrocyte-rosette formation test (E-rosette) was carried out according to Gengozian et al. (2002) for estimation of T-lymphocytes.

The concentration of IFN-γ was estimated according to the procedure provided by the manufacturer (Biosource, Eumpe, S.A). The biosource IFN-γ is a solid phase enzyme amplified sensitivity Immune assay (EASIA) performed on a microtiter plate.

The concentration of IL-4 in patient's sera was carried out according to the procedure provided by the manufacturer (Biosource, Eumpe, S.A). The assay is based on an oligoclonal system in which a blend of monoclonal antibodies (MAbs) directed against distinct epitopes of IL-4 are used. IL-4 that are present in the sample reacts with captured monoclonal antibodies (MAB1) coated on the microtiter well and with a monoclonal antibody (MAB2) labeled with horseradish peroxidase (HRP).

The concentrations of immunoglobulin G and M (IgG and IgM) were estimated using the technique of single radial immunodiffusion test (SRID) (Biomaghrab, Tunisia) according to Lewis et al. (2001).

Statistical analysis

The mean, standard deviation (SD), and analysis of variance (ANOVA) tests were calculated according to Cochran (1974).

RESULTS AND DISCUSSION

Results of blood culture revealed that positive bacterial blood cultures (bacteremia) were observed in 10 diabetic patients (28.57%) with type I, and 20 patients (22.2%) with type II (Table 1). These results indicated the

Table 1. Distribution of bacteremia among diabetic patients and controls.

Patient group	Total number	Number of bacteremia (%)	Number of non-bacteremia (%)
Diabetes mellitus type I	35	10 (28.57)	25 (71.43)
Diabetes mellitus type II	90	20 (22.2)	70 (77.8)
Total	125	30 (24)	95 (76)
Control subject	55	0	55 (100)

Table 2. Gram-positive and Gram-negative bacteria isolated from diabetic patients.

Bacterium species	Number of isolates (%)
Gram positive	
<i>Staphylococcus epidermidis</i>	13 (61.90)
<i>Streptococcus mitis</i>	4 (19.05)
<i>Staphylococcus aureus</i>	3 (14.29)
<i>Bacillus cereus</i>	1 (4.76)
Total	21 (100)
Gram negative	
<i>Klebsiella pneumoniae</i>	4 (44.44)
<i>Escherichia coli</i>	2 (22.22)
<i>Citrobacter freundii</i>	1 (11.11)
<i>Proteus mirabilis</i>	1 (11.11)
<i>Pseudomonas aeruginosa</i>	1 (11.11)
Total	9 (100)

presence of bacteremia in both types of diabetic patients which were also obtained by other investigators (Akbar, 2000; Cisterna et al., 2001).

Results of distribution of bacterial isolates according to type of Gram staining, revealed that out of 30 bacterial isolates recovered from patients with diabetes, 21 (70%) were Gram-positive bacteria while 9 (30%) were Gram-negative bacteria. The predominance of Gram-positive bacterial isolates in blood cultures of diabetic patients in the present study was in accordance with findings of several authors who mentioned that Gram-positive bacteria were predominant agents of bacteremia in diabetic patients (Thomson et al., 2004; Moutschen, 2005).

Results of identification of Gram-positive and -negative bacterial isolates based on cultural characteristics and biochemical properties of these isolates revealed that these isolates were of different bacterial species (Table 2). *Staphylococcus epidermidis* was the major Gram-positive species (61.90%), while *Bacillus cereus* (4.76%) was the less commonly isolated Gram-positive species in diabetic bacteremia. These results agreed with VonEiff et al. (2001) who reported that *S. epidermidis* was the predominant agent of bacteremia in immunocompromized patients.

Klebsiella pneumoniae was the predominant species among Gram-negative bacterial species (44.4%), while the other bacterial species such as *E. coli*, *Citrobacter*

freundii, *Proteus mirabilis* and *Pseudomonas aeruginosa* were isolated in low percentages. These findings agreed with the results of Oni et al. (2000) who mentioned that *Klebsiella* spp. represented 43% of all Gram-negative bacterial isolates recovered from bacteremia in diabetic patients. At the same manner, Al-Muslemawi (2007) showed that *Citrobacter freundii* may cause bacteremia in immunocompromized patients including patients with D.M.

All Gram-positive bacteria isolated in the present study were non-capsule producers. Gram-negative bacterial isolates were also non-capsule producers except *K. pneumoniae* isolates (Table 3) which exhibited a large and clear capsule when examined by negative staining method.

All *S. aureus* isolates were able to produce coagulase which is considered a virulence factor for pathogenicity of these bacteria by clumping the fibrin around the bacteria (Forbes et al., 2007). Possibly, coagulase could provide an antigenic disguise if it clotted fibrin on the cell surface or could make the bacterial cells resistant to phagocytes or tissue bacterial target (Humphreys, 2004).

Table 3 showed that *S. aureus*, *E. coli*, and *P. aeruginosa* isolates expressed β -hemolysis on blood agar medium while all *Streptococcus mitis* (4:4) isolates exhibited α -hemolytic pattern, showing a greenish zone around the bacterial colonies. On the other hand,

Table 3. Virulence factors of bacterial isolates recovered from diabetic patients.

Bacterial isolate	Coagulase	Lipase	Haemolysis	Gelatinase	Capsule formation	*CF-I	*CF-III	Bacteriocin production
<i>Staphylococcus aureus</i> 1	+	+	β	+	-	+	+	+
<i>S. aureus</i> 2	+	+	β	+	-	+	+	+
<i>S. aureus</i> 3	+	+	β	+	-	+	+	+
<i>Staphylococcus epidermidis</i> 1	-	-	γ	-	-	+	+	-
<i>S. epidermidis</i> 2	-	-	γ	-	-	+	+	-
<i>S. epidermidis</i> 3	-	-	γ	-	-	+	+	+
<i>S. epidermidis</i> 4	-	-	γ	-	-	+	+	+
<i>S. epidermidis</i> 5	-	+	γ	-	-	+	+	+
<i>S. epidermidis</i> 6	-	+	γ	-	-	+	+	-
<i>S. epidermidis</i> 7	-	-	γ	-	-	+	+	-
<i>S. epidermidis</i> 8	-	-	γ	-	-	+	+	-
<i>S. epidermidis</i> 9	-	-	γ	-	-	+	+	-
<i>S. epidermidis</i> 10	-	+	γ	-	-	+	+	-
<i>S. epidermidis</i> 11	-	-	γ	-	-	+	+	-
<i>S. epidermidis</i> 12	-	-	γ	-	-	+	+	+
<i>S. epidermidis</i> 13	-	-	γ	-	-	+	+	+
<i>Streptococcus mitis</i> 1	-	-	α	-	-	+	+	-
<i>S. mitis</i> 2	-	-	α	+	-	+	+	+
<i>S. mitis</i> 3	-	-	α	-	-	+	+	+
<i>S. mitis</i> 4	-	-	α	-	-	+	+	+
<i>Bacillus cereus</i>	-	-	γ	+	-	+	+	+
<i>Klebsiella pneumoniae</i> 1	-	+	γ	-	+	+	+	+
<i>K. pneumoniae</i> 2	-	+	γ	-	+	+	+	+
<i>K. pneumoniae</i> 3	-	+	γ	-	+	+	+	+
<i>K. pneumoniae</i> 4	-	-	γ	-	+	+	+	+
<i>Escherichia coli</i> 1	-	+	β	-	+	+	+	+
<i>E. coli</i> 1	-	+	β	-	-	+	+	+
<i>Citrobacter freundii</i>	-	+	γ	-	-	+	+	+
<i>Proteus mirabilis</i>	-	+	γ	+	-	+	+	-
<i>Pseudomonas auroginosa</i>	-	+	β	+	-	+	+	+

*CF: colonization factor antigens.

Staphylococcus epidermidis (13:13) isolates and each of *B. cereus*, *C. freundii* and *Proteus mirabilis* isolates were γ-hemolytic (non hemolytic).

The production of hemolysin by *S. aureus* is well known and considered as a main virulence factor for these bacteria and it is associated with

increased severity of infections (Humphreys, 2004).

In this study, gelatin was used as a protein for

Table 4. Antibiotic resistance of gram-positive isolates recovered from diabetic patients.

Antibiotic	Bacterial isolate (Number)							
	<i>S. epidermidis</i> (13)		<i>S. aureus</i> (3)		<i>S. mitis</i> (4)		<i>B. cereus</i> (1)	
	R	S	R	S	R	S	R**	S*
Amoxicillin	0	1	3	1	2	1	11	2
Oxacillin	0	1	3	1	2	1	11	2
Cefoxitin	0	1	1	3	1	2	3	10
Cefotaxime	0	1	0	4	0	3	0	13
Amikacin	0	1	2	2	1	2	0	13
Tetracycline	0	1	0	4	1	2	3	10
Doxycycline	0	1	1	3	1	2	4	9
Ciprofloxacin	1	0	2	2	0	3	0	13
Nalidixic acid	1	0	2	2	3	0	5	8
Trimethoprim-sulphamethazole	0	1	1	3	0	3	0	13

* Sensitive; ***Resistant.

detection of protease activity in bacterial isolates. In Gram-positive bacteria, *S. epidermidis* (13:13) isolates were negative for this factor while *S. aureus* isolates (3:3) were positive for this test. In addition to that, a single isolate of *B. cereus* was able to produce gelatinase. One isolate of *Streptococcus mitis* was also positive for this test.

A single isolate each of *P. mirabilis* and *P. aeruginosa* were positive for gelatinase enzyme, while all isolates of *K. pneumoniae* and *E. coli* were negative for this factor (Table 3). Gelatinase is a proteases enzyme considered as a potential virulence factor of many microorganisms because of its ability to breakdown immunoglobulin and complement components that make up the host defenses against microbial infections and therefore, enable the pathogens to invade the host tissues (Travis et al., 1995).

All *S. aureus* (3:3) isolates were positive for lipase enzyme production, while only (4:13) isolates of *S. epidermidis* were positive for this factor. A single isolate of *B. cereus* and all isolates of *S. mitis* were also negative for this enzyme however, most Gram-negative isolates (8:9) were positive for this test. Host cell membranes have lipids in their components and lipase enzyme will destroy this element and aid the pathogen to penetrate the host tissue to develop the infections (Lisa et al., 1994).

Twenty (66.67%) of the total bacterial isolates recovered from diabetic patients were able to produce bacteriocin by cup assay method and form a clear inhibition zone (12 to 22 mm) on solid medium. These findings are in agreement with the results obtained by many researchers (Al-Qassab and Al-Khafaji, 1992; Al-Charrakh, 2005) who found that cup assay method was the best method used for detection of bacteriocin-production by isolates of *Lactobacilli*, and *Klebsiella pneumoniae* respectively. The importance of bacteriocin for virulence and pathogenicity of bacteria is controversial. Bacteriocin is essential for virulence and pathogenicity of

Enterococcus in septicemia (Hancock and Gilmore, 2000) because it was found that cytolysin of *Enterococcus faecalis* (possess both hemolysin and bacteriocin activities) promotes the appearance of this bacteria in the blood thus indicating that the bacteriocin is essential for virulence of these bacteria in blood stream infections.

Antibiotics resistance of bacterial isolates

Results in (Table 4) show the antibiotic resistance of Gram-positive bacterial isolates recovered from diabetic patients. Most isolates of *S. epidermidis* (11:13) were highly resistant to amoxicillin and oxacillin, but they were highly sensitive to cefotaxime, amikacin, ciprofloxacin and trimethoprim-sulphamethazole. They also showed low levels of resistance to nalidixic acid, tetracycline and cefoxitin. Humphreys (2004) reported that the main mechanism of resistance of *S. epidermidis* to β -lactams is mediated by β -lactamase production. Results revealed that *S. epidermidis* had the highest number of multi-drug resistant isolates, and these findings are in agreement with those obtained by other investigators (Mahmood, 2001; VonEiff et al., 2001). *S. epidermidis* may act as a reservoir for resistance which can be transferred to *S. aureus*. All isolates of *S. aureus* (3:3) were resistant to nalidixic acid. Two of these isolates were resistant to amoxicillin and oxacillin, and only one isolate was resistant to cefoxitin, amikacin and tetracycline. These results are similar to the findings obtained by Brook et al. (1981) who showed that *S. aureus* isolates were resistant to amoxicillin and less resistant to cefotaxime. A further significant increase in the rate of oxacillin resistance, as was observed in the early 1990s, was not registered.

However, one may speculate that these rates would have been even higher if we had also included isolates from body sites other than blood, as was done in former studies (Kresken and Hafner, 1999). Three isolates of *S.*

Table 5. Antibiotic resistance of gram-negative isolates recovered from diabetic patients.

Antibiotic	Bacterial isolate (Number)									
	<i>K. pneumoniae</i> (4)		<i>E. coli</i> (2)		<i>C. freundii</i> (1)		<i>P. mirabilis</i> (1)		<i>P. aeruginosa</i> (1)	
	S*	R**	S	R	S	R	S	R	R	S
Amoxicillin	1	0	1	0	0	1	1	1	4	0
Oxacillin	1	0	1	0	0	1	2	0	3	1
Cefoxitin	1	0	0	1	0	1	1	1	2	2
Cefotaxime	0	1	0	1	0	1	0	2	1	3
Amikacin	1	0	1	0	1	0	2	0	4	0
Tetracycline	0	1	0	1	0	1	1	1	1	3
Doxycycline	1	0	0	1	0	1	1	1	3	1
Ciprofloxacin	1	0	1	0	1	0	2	0	3	1
Nalidixic acid	1	0	0	1	1	0	1	1	3	1
Trimethoprim-sulphamethazole	1	0	0	1	0	1	0	2	1	3

* Sensitive; **Resistant.

mitis were resistant to amoxicillin and oxacillin, with two resistant to amikacin, ciproflaxin and nalidixic acid, and only one isolate was resistant to cefoxitin, doxycycline and trimethoprim-sulphamethazole (Table 4).

The results agreed with Carratala et al. (1995) who mentioned that bacteremia due to viridans streptococci were highly resistant (77%) to penicillin products. Among the streptococcal species, the viridans streptococci may be the most important pathogens causing bacteremia and sepsis in immunocompromized patients (Patrick, 1999). This problem is exacerbated by the emerging resistance of streptococci to antimicrobial agents commonly used for empirical and prophylactic treatments in those patients. The results also showed that the single isolate of *B. cereus* was sensitive to most antibiotics tested in the current study, and this result was confirmed by many researchers who found that *Bacillus spp.* isolates were highly sensitive to most antibiotics used in their studies (Weber et al., 1988).

Table 5 shows the results of antibiotic resistance of Gram-negative bacterial isolates. All isolates of *K. pneumoniae* were resistant to amoxicillin and amikacin with three being resistant to oxacillin, doxycycline, ciproflaxin and nalidixic acid, and two were resistant to cefoxitin and only one isolate was resistant to cefotaxime, tetracycline and trimethoprim-sulphamethazol. These results were in agreement with the reports published by some workers (Al-Charrakh et al., 2011) from Iraq and another researcher (Jarlier et al., 1996) in France who mentioned that *K. pneumoniae* was highly resistant to amoxicillin and ampicillin by the production of β -lactamases that render these isolates unsusceptible to most β -lactam antibiotics.

Two isolates of *E. coli* recovered in the present study were resistant to oxacillin, amikacin and ciproflaxin but they were sensitive to cefotaxime and trimethoprim-sulphamethazole (Table 5). This resistance could be

interpreted depending on the fact that many strains of *E. coli* have acquired plasmids conferring resistance to one or more than one type of antibiotics, therefore antimicrobial therapy should be guided by laboratory result test of sensitivity. However, the sensitivity of *E. coli* isolates agreed with local reports in Iraq by several researchers (Al-Muhanna, 2001; Al-Hamawandi, 2005) who showed that all isolates of *E. coli* were sensitive to cefotaxime and trimethoprim-sulphamethazole. The single isolate of *Citrobacter freundii* was resistant to amikacin although this antibiotic is not used widely in the treatment in Iraq while the single isolate of *P. mirabilis* was resistant to amoxicillin, oxacillin, amikacin and ciproflaxin (Table 5). The single isolate of *P. aeruginosa* was resistant to most antibiotics tested and this result agreed with results reported by several investigators (Mahmoud, 2001; Kiska and Gilligan, 2003) who showed that *P. aeruginosa* strains isolated from blood-stream infections were resistant to 3rd generation cephalosporins and quinolones.

Immunological parameters

Table 6 shows the results of E-rosette test. The mean value of E. rosette that was positive for T-lymphocytes in both type I and II D.M was less than control subjects. T-cell counts decreased significantly ($p < 0.05$) in both types of diabetic patients, whereas the mean difference between the two types of diabetic patients was non-significant ($P > 0.05$). These results indicate the effect of hyperglycemia on the proliferation and function of T-cells that leads to immunosuppression of cellular immunity in diabetic patients. These results agreed with many reports which pointed that the main causes responsible for subnormal T-cell levels are acquired immune deficiency disorders including D.M., acute viral infections, and congenital immunodeficiency disease (Kotton, 2004).

Table 6. The T-cells count estimated and IFN- γ concentration for diabetic patients and control subjects.

Test group	T-cells count		IFN- γ concentration	
	Mean value %	Standard error	Mean I.U/ml	Standard error
Diabetes mellitus type I	9.1	1.16	0.285	0.085
Diabetes mellitus type II	10.63	0.817	0.313	0.060
Control subjects	22.55	1.16	0.860	0.085

Table 7. IL-4 concentration and Mean values of the IgG and IgM concentrations in the serum of diabetic patients and control subjects.

Test group	IL-4 concentration		IgG and IgM concentrations	
	Mean (pg/ml)	Standard error	Mean value of IgG (mg/dL)	Mean value of IgM (mg/dL)
Diabetes mellitus type I	7.050	2.523	1674.45	145.78
Diabetes mellitus type II	7.703	1.784	2095.86	177.64
Control subjects	13.650	2.523	1269.51	112

Decreasing T-cells count greatly contributes to impaired cell mediated immunity (Moretti, 1992). This impairment is due to the supporting role of T-cells activation in both arms of immune responses; cellular and humoral (T_{H1} and T_{H2} cells); in addition to its direct roles by directly killing foreign invaders by cytotoxic T-lymphocytes (Abbas et al., 2000).

Several immunological abnormalities related to D.M. such as depletion of T-cells, defective NK cells and insulinopenia-induced enzymatic defect have often been proposed to inhibit energy-requiring functions of phagocytes and lymphocytes (Moutschen et al., 1992).

Results also revealed that IFN- γ decreased significantly ($p < 0.05$) in both types of diabetic patients as compared with control subjects (Table 6). These results indicate the presence of reduced cellular immunity in diabetic patients which agreed with the results obtained by several authors (Kukreja et al., 2002; Tsiavon et al., 2005) who stated that IFN- γ decreased significantly in diabetic patients. However, in the present study there was no significant ($p > 0.05$) differences between the two types of D.M.

IFN- γ is secreted from T_{H1} lymphocyte, NK cells and macrophage, therefore any defect in these cells leads to decreasing IFN- γ production that reflects impaired host resistance in diabetic patients due to a dysregulation of the cytokine network. IFN- γ enhances the microbicidal function of macrophages by stimulating the synthesis of reactive oxygen intermediates and nitric oxide (NO). It promotes the differentiation of naïve $CD4^+$ T-cells to the T_{H1} subset and inhibits the proliferation of T_{H2} cells beside activating neutrophils and stimulates the cytolytic activity of NK cells (Doan et al., 2005).

Between-group comparisons of IL-4, IgG and IgM are presented in Table 7. IL-4 showed significant between group differences ($p < 0.05$), with no difference detected

between type I and type II DM (mean of 7.05 and 7.70 pg/ml respectively), but both types of DM were significantly different from controls (mean of 13.65). These results agreed with Mayer et al. (1999) and Kukreja et al. (2002) who mentioned that there was no significant reduction in the levels of IL-4 among diabetic patients as compared with controls. These results expressed that T_{H2} response is slightly affected in DM as compared with T_{H1} response.

Regarding levels of IgG and IgM in diabetic patients and control subjects, the results revealed that the levels of IgG increased significantly ($p < 0.05$) in both types of diabetic patients, while IgM levels increased significantly ($P < 0.05$) in type II and non-significantly ($p > 0.05$) in type I as compared with control subjects (Table 7). These findings agreed with those obtained by Gorus et al. (1998) who mentioned that the mean levels of IgM and IgG were higher in both types of diabetes mellitus patients.

Changes in total immunoglobulin concentrations were largely reversed under insulin therapy which may reflect exposure to environmental triggers, such as viral infections or to insulinopenia prior to clinical disease onset (Gorus et al., 1998). Alteration in humoral immune response was less in cellular immunity during immunosuppression process. Therefore levels of IgG and IgM in diabetic patients are higher than in normal persons but not like levels of IgG and IgM in persons suffering from microbial infections without diabetes. Some reports mentioned that diabetes might exhibit hypergammaglobulinemia (Al-Ardawi et al., 1994).

The results expressed in Table 8 show the relationship between bacteremia and immunological parameters studied in type I and II diabetic patients. E-rosette value, which is used as a marker for T-cells count, decreased significantly ($p < 0.05$) in bacterimic diabetic patients as

Table 8. Relationship between bacteremia and immunological parameters in diabetic patients.

Test group	Total no. (%)	Immunological parameter				
		E-rosette* (%)	IFN- γ * (I.U/ml)	IL-4* (pg/ml)	IgG* (mg/dL)	IgM* (mg/dL)
Bacteremia	30 (24)	8.217**	0.109 **	5.478	1865.24	168.5
Non-bacteremia	95 (76)	15.246 **	0.577 **	10.458	1626.996	138.47
Total No.	125 (100)					

*The mean value is calculated for both types of D.M. with bacteremia..**The mean difference is significant at 0.05 level.

compared with non bacteremic diabetic patients. Mean value of IFN- γ concentration in diabetic patients with bacteremia was 0.109 I.U./ml, while in diabetes mellitus patients without bacteremia, it was 0.577 I.U./ml (Table 8). These results show significant decrease ($p < 0.05$) in IFN- γ concentration in diabetic patients with bacteremia as compared with non bacteremic diabetic patients. Therefore, decreasing IFN- γ concentration leads to increase likelihood of bacteremia in diabetic patients (Abbas et al., 2000). Microbial products stimulate macrophages to produce tumor necrosis factor alpha (TNF- α) and IL-12. Together, these two cytokines then stimulate NK cells to produce IFN- γ (Bancroft et al., 1991). This cytokine plays an important role in the growth and differentiation of cytotoxic T-cells, activates NK cells and acts as a B-cell maturation factor. It regulates immunoglobulins (Ig) isotype production (Snapper and Paul, 1987; Murray, 1988). Concerning levels of IL-4 concentration in bacteremic and non-bacteremic diabetic patients results, the study revealed that levels of IL-4 decreased non-significantly ($p > 0.05$) in bacteremic diabetic patients as compared with non bacteremic diabetic patients. IL-4 is produced by TH₂ type of CD⁴⁺ T-lymphocytes, following activation by the antigen binding to the T-cell receptor and also produced by activated mast cells and basophiles (Abbas and Lichtman, 2007). IL-4 deregulates the production of IFN- γ by TH₁ CD⁴⁺ T-lymphocytes, on the B-cells (Banchereau, 1990).

The levels of IgG and IgM in bacteremic diabetic patients were 1865.24 and 168.5 mg/dL respectively, while in non bacteremic patients they were 1626.996 and 138.47 mg/dL, respectively. These results indicated non-significant increase ($p > 0.05$) in immunoglobulin levels in bacteremic diabetic patients as compared with non bacteremic diabetic patients.

Immunoglobulin increased during courses of bacteremia by 10 to 15 fold than in normal patients. Thus, the non significant increased levels of immunoglobulin in bacteremia diabetic patients is not high enough for the level of protection from infections. Therefore, there are non-significant differences between patients with bacteremia and controls without bacteremia. This may be due to shortage in the signaling of TH₂ required for B-cell proliferation and differentiation into immunoglobulin-secreting cells (plasma cells) (Rich et al., 2003).

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