BACTERIAL PROFILE IN PATIENTS WITH DIABETIC FOOT INFECTIONS AND ITS ASSOCIATION WITH TNF-α

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Abstract

Diabetes mellitus (Type 2) is a crucial health problem, that is rapidly developed globally. One of the most common diabetic complications is diabetic foot infection that occurs from a complicated interplay between a number of peril elements such as neuropathy, peripheral vascular disease, a foot malformation and a trauma. The study aimed to investigate the bacterial causes of diabetic foot ulcer and determine the antibiotic resistance pattern of the bacterial isolates. In addition to that serum levels of TNF-α was evaluated in diabetic patients (type 2) with foot infections. The study involved 95 wound samples were taken from diabetic patients (type 2) with foot infections of both sexes, age ranged between 40-80 years. Bacteria isolates and identified by standard laboratory techniques. Antibiotic susceptibility test to commonly used antibiotics was done according to Kirby-Bauer method. Serum levels of TNF-α was measured in diabetic (type 2) patients with foot infections and control group by using ELISA test. Staphylococcus aureus most common pathogens isolated from diabetic patients followed by P. aeruginosa and E. coli. In this study bacterial pathogens showed resistance to nearly all of the antibiotics. Imipenem was the most efficient antibiotics against tested isolates. Higher levels of TNF-α were detected in diabetic patients (277.44 pg/ml) compared to control groups (70.13 pg/ml). S. aureus was the most common isolated bacteria. There are increasing level of TNF-α in diabetic patients with foot infections.

Keywords: Diabetes mellitus, Staphylococcus aureus.

Introduction

Type (2) diabetes milieus is the most common kind of diabetes, accounting for 90-94% of all diabetes. It usually develops after the age of 40, but it may occur at any age. It is more prevalent among people who are older, with sedentary a life, overweight, or have a family history of the disease (Soní, 2013). Diabetes mellitus (Type 2) or non-insulin dependent diabetes mellitus (NIDDM) is characterized by impaired beta cell function, insulin resistance which may be combined with relatively reduced insulin secretion and is often associated with other metabolic abnormalities (Al-Tu’ma et al., 2011).

Patients with diabetes possess a greater incidence and intensity of several common infections including pulmonary, urinary and soft tissue infections. Foot infections one of the most common bacterial infections noticed in patients with DM in clinical practice recorded for up to 20% of diabetes-associated hospital admittance (Salihii and Jumaah, 2013). When the skin is broken, the hidden tissues are subjected to pathogenic organisms colonization (Mutluoglu et al., 2011). The resulting wound infection may begin externally, but with delay in medication and weaken body protection mechanisms, it can distributed to the subcutaneous tissues and to even deeper structures. These infections and their squeal are the majority cause for lower-limb amputation (Raheem et al., 2012). Most of DFIs are multimicrobial with a blend of aerobic and an aerobic organisms. The treatment of infection in diabetic patients becomes hard because of antibiotic resistance to the frequently used antibiotics as a result of misuse of particular antibiotics (Kamel et al., 2014).

The increased susceptibility of diabetic patients to infections and spoiled wound curative is due to decline vascularity to the lower limb, autonomic dysfunction (Singh and Sridha, 2015), defects in both cell-mediated immunity (CMI) and humorallimmunity; also immune senescence, which happens as a result of aging and which mostly influence CMI (Rajagopalan, 2005). Diabetic foot wounds are marked by a continuing and unbalanced inflammatory state, an enhanced producing and liberate of pro-inflammatory cytokines such as (IL-1β, IL-6 and TNF-α) that causes disruption the equilibrium between proinflammatory and anti-inflammatory cytokines (Cory et al., 2015). Diabetic patients have also exhibited a remarkable up regulation insserum TNF-α through high blood glucose episodes compared with a comparatively, little change seen in normal patients (Gordin et al., 2008).

The aim of this study was to determine the common bacterial causes of diabetic foot infections and determine the antibiotic susceptibility pattern of the
bacterial isolates. 2- Evaluate the serum level of TNF-α in diabetic patients (type 2) with foot lesions.

Material and Methods

Bacterial Samples Collection and Diagnosis

The study was carried out on 95 diabetic patients (type2) with foot infections of both sexes with age ranged between 40-80 years who tended to the Merjan medical city and Al-Hilla teaching hospital through a period of six months. Samples involved pus or discharges from the ulcers abase and debrided necrotic tissues were collected by deep swab. The samples were inoculated onto the blood agar and MacConkey’s agar, then incubated aerobically at 37ºC for 48 hours. For isolation of anaerobes bacteria, the specimens were incubated in an anaerobic chamber at 37 ºC and examined at 48 hours and 96 hours after incubation. All bacterial isolates were diagnosis by gram's staining and conventional biochemical tests (Collee et al., 1996).

Antimicrobial Susceptibility Testing

Antibiotics sensitivity testing was conducted by Kirby Bauer's disc diffusion method and access according to recommended National Committee- for Clinical Laboratory Standards (CLSI) guidelines. The used antibiotics were: Cefotaxime, Cephalexin, Cefazidime, Ciprofloxacin, Cloxacillin, Carbencillin, pipracillin, Meropenem, Gentamicin, Augmentin, Lincomycin, Kanamycin, Metronidazole (Forbes et al., 2007).

Estimation the Level of TNF- α

Five ml of venous blood was collected in test tube from (66) diabetic patients (type II) with foot lesion and (10) healthy control individual for estimation TNF-α level. The blood was leaved to clot and the serum was separated-by centrifugation (2500 rpm for 10-min). Sera samples were dispensed into tubes and stored at -20°C until used. The serum level of (TNF- α) in diabetic patients and control group was examined using ELISA test (kits of Boster, USA).

Statistical Analysis

Statistical analysis was conducted. Data were presented as mean and standard deviation or number and percentage as appropriate. The Chi square test was used to analyze the significance of the results. P value <0.05 was considered significant.

Results and Discussion

Isolation and Identification of Bacterial Isolates

A total of 95 hospitalized patients-with diabetic foot infections of both sexes, age ranged between 40-80 years were examined through period of six months. The results of this study showed positive growth from only 80 (84.2%) patients, whereas 15(15.8%) patients no bacteria was isolated (Table1). Similar results was reported by Manisha et al. (2012) in his study who found that out of 125-specimens,-108 (86.4%) specimens exhibited bacterial growth were isolated while 17 (13.65%) specimens did not show any growth. The negative culture from infected foot could be attributed to the usage of local antibiotics and antiseptic during wound dressing that decrease the number of isolated bacteria.

Diabetic foot ulcers are common and serious complications of persistent DM. In parallel with a grow prevalence of this disease, the prevalence of foot infections are elevating worldwide (Raheem et al., 2017).

<table>
<thead>
<tr>
<th>Table 1 : Percentage of positive and negative culture</th>
</tr>
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<tbody>
<tr>
<td>No. of samples</td>
</tr>
<tr>
<td>95</td>
</tr>
</tbody>
</table>

Single type of bacteria was found in foot infections and polymicrobial infection (i.e. infection with different type of bacteria) was also detected. Many studies support this findings (Akwah et al., 2015; Reghu et al., 2016). Among 80 bacterial isolates, 68 were aerobic, while 20 were anaerobic bacteria (Table 2). Similar findings were recorded by (Haldar et al., 2017; Garg et al., 2017).

Many studies support the result of the present study that G-ve bacteria exhibited high prevalence in diabetic-foot infection (Tiwari et al., 2012; Sona et al., 2016; Khare et al., 2017).

In this study, S. aureus was the predominant and commonest pathogen, followed by P. aeruginosa and E. coli. The bacterial types isolated-from the diabetic foot infections were included in-(table-.2).

<table>
<thead>
<tr>
<th>Table 2 : Bacterial-species isolated from diabetic foot infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of bacteria</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Aerobic</td>
</tr>
<tr>
<td>S. aureus</td>
</tr>
<tr>
<td>S. epidermidis</td>
</tr>
<tr>
<td>Streptococcus pvogenes</td>
</tr>
<tr>
<td>E.coli</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
</tr>
<tr>
<td>K. pneumonia</td>
</tr>
<tr>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>Enterobacter Spp.</td>
</tr>
<tr>
<td>Anaerobic bacilli</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Similar results recorded in Jaddue-and Al-Kaisi., (2008)-study, that S. aureus was most common bacteria,
while *P. aeruginosa* and *E. coli* were the second common bacteria obtained from diabetic foot infections. Many studies like (Muter et al., 2012; Tamalli et al., 2015; Smith et al., 2016; Ibrahim et al., 2016) support the result of the this-study that *S. aureus* was the most frequent kind of bacteria seen in diabetic foot infections. *S. aureus* is the most vital isolated pathogen of skin-infections in generic and in manageable -diabetic foot infections.

Diabetic patients usually own persistent non curative foot ulcers by cause of several critical such as elevated plantar pressures, neuropathy and peripheral arterial disease, the risk by high levels of blood sugar in the diabetic patients which destroyed blood vessels, induced them to grow thick and leakage, this make vessels lesser capable to provide the body, exclusively the skin with blood to survive health, the result of low circulation induced ulcers, exclusively those found in the feet, like persist long-standing ulcers. So a board spectrum of bacteria can promote infection in those patients (Jeber and Saeed, 2013). An understanding of the bacterial causes of diabetic foot-infections is essential un-giving antibiotic choice and associated culture result with suitable treatment (Jaddue et al., 2008).

**Distribution of Diabetic Foot Patients According to the Gender and Age**

Regarding the gender, the results revealed that most of diabetic foot patients are men 48 (60%) (Table 3) (P = 0.074). Male predominance was seen in other studies (Chakraborty and Mukherjee, 2015; Karmaker et al., 2016; Gangania and Singh, 2016; Gopi et al., 2017). This may be as results of higher level of outdoor activity among males compared to females. Also male is subjected more to trauma, in addition to smoking and alcohol drinking which is seen more with male (Tamalli et al., 2015).

**Table 3** : Distribution of patients according to the gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>48</td>
<td>60 %</td>
</tr>
<tr>
<td>Female</td>
<td>32</td>
<td>40%</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>100%</td>
</tr>
</tbody>
</table>

χ²=3.200, P value=0.074

The results of current study shows that most of diabetic foot patients belongs to age group ranging between 61-70 than other age (table 4) (P = 0.019). This results confirmed by (Dwedar et al., 2015) who found that maximum number of patients belong to age group 60 to 65 years. Mahmood, (2007) also recorded that major of diabetic foot patients with age above sixty years.

The predominance of diabetic foot ulcers in this age range may be due to the fact that patients in this age range have nutritional deficiencies and decreased immunity. In addition, the elderly have-lived for a longer time with diabetes mellitus-than the younger patients by that forming-them more susceptible to the complexity and foot-ulceration (Akwah et al., 2015).

**Table 4** : Distribution of patients according to the age

<table>
<thead>
<tr>
<th>Age group</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>40-50</td>
<td>15</td>
<td>18.75%</td>
</tr>
<tr>
<td>51-60</td>
<td>23</td>
<td>28.75%</td>
</tr>
<tr>
<td>61-70</td>
<td>30</td>
<td>37.5%</td>
</tr>
<tr>
<td>71-80</td>
<td>12</td>
<td>15%</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>100%</td>
</tr>
</tbody>
</table>

χ²=9.900, P value=0.019

**Antibiotic Susceptibility Pattern**

The sensitivity of the isolated bacteriato traditionally used-antibiotics was detected by Bauer-Kirby method. Majority off *S. aureus* and *S. pygenes* bacteria were-sensitive to imipenem, ceftriaxone, ceftazidime and amoxicillin-clavulanic-acid and gentamycin. All isolates of *S. pygenes* were susceptible to amikacine, but *S. aureus* showed less sensitivity to gentamycin and amikacine (Fig-1). Our finding were compatible with Ratemo, (2014) study who recorded that *S. aureus* was susceptible to imipenem, amoxicillin-clavulanic acid, ceftazidime and ceftriaxone Banoo et al. (2012) in his study showed that gram-positive organisms sensitive to amoxicillin/clavulanic acid and they were highly resistant gentamicin, while Gangania and Singh, (2016) stated that cephalosporins and majority of the aminoglycosides were effective against gram positives bacterial isolates.

Antimicrobial susceptibility results showed that all *K. pneumoniea, P. vulgaris* and *Enterobacter Spp* isolates were sensitive to amikacine, while it’s has variable susceptibility to ceftriaxone, ceftazidime and gentamycin and full resistance to amoxicillin-clavulanic acid.

*E. coli* were most sensitive to toceftazidime (90%), ceftriaxone (90%), gentamycin (70%), while the sensitivity to amoxicillin-clavulanic acid was (50%) and to amikacine was (10%). The results off current study in accordance with results of Regha et al. (2016) that the sensitivity of *P. vulgaris* to amikacine was 100%. Sekhar et al. (2014) stated that most gram-negative isolates including ESBL producing strains of *Proteus* were highly sensitive to amikacine.
broad spectrum antibiotics in our hospital result in resistance to ceftriaxone, with a sensitivity of 88.9%.

Similarly to our results, E. coli was found unsusceptible to the most of tested antibiotics, with the exception of gentamicin and imipenem. Also, it’s found that *proteus, E. coli* and *Pseudomonas* showed sensitivity to ceftazidime (Banashankari et al., 2012).

Others studies like Citron et al. (2007) revealed that *Enterobacteriaceae* family were susceptible to imipenem, ceftazidime, aminoglycosides, while amoxicillin-clavulanate were the least effective antibiotic against gram negative organisms. It is necessary to acknowledge that some gram-negative bacteria from the *Enterobacteriaceae* family possess the capacity to produce highly-active β-lactamase enzymes, forming them susceptible to β-lactam and cephalosporins antibiotics (Perim et al., 2015).

*P. aeruginosa* exhibited resistance to amoxicillin-clavulanic acid (83.3%), gentamycin (75%), amikacine (75%) and ceftriaxone (66.6%). Our results is confirmed by (Tamalli et al., 2015). Chaudhry et al. (2016) stated that *P. aeruginosae* isolates were highly resistant to the most of tested antibiotics.

This increasing multidrug resistant organisms occurrence is a potential peril component in treatment of diabetic foot infections which may promote complexity such as systemic toxicity, gangrene development and amputation of lower parts (Manisha et al., 2012; Mathangi and Prabhakaran, 2013). Its responsible for the increased time of hospitalisation, cost of treatment and mortality of the diabetic-patients (Umadevi et al., 2011).

In present study, most bacterial isolates were sensitive to imipenem (100%), Similar result was recorded by others studies (Perim et al., 2015; Akwah et al., 2015; Haldar et al., 2017) that imipenem was the most active antibiotic against gram positive and gram negative bacteria; therefore, this antibiotic could be useful for usage in empirical treatment. Although in last years, there has been an increasing in the resistance to imipenem and cephalosporins probably due to their misuse of these antibiotics (Gopi et al., 2017).

Antibiotic sensitivity test of anaerobic organisms showed that most isolates were sensitive to imipenem (100%), metronidazole (100%), amikacine (90%), amoxicillin clavulanic acid (75%), cefazidime (65%), ceftriaxone (60%), gentamycine (50%). Similar findings were recorded by Banoo et al. (2012), that all the anaerobes were-sensitive to metronidazole and imipenem. Haldar et al. (2017) also mentioned that imipenem and metronidazole had lowest resistance rates against anaerobic organism.

The increasing rates of antibiotic resistance found in this study may be because of wide spread-usage of broad spectrum antibiotics in our hospital result in selective survival benefit of pathogens (Ibrahim et al., 2016; Anvarinejad et al., 2015). Also, antibiotic resistance can be partially ascribe to the capacity of these bacteria to make small colony variants after the exposed to environmental stressors or antibiotics. These variants more flexible and considered a survival mechanism used by number bacterial pathogen, such as *S. aureus* to resist changes in the environmental conditions. A late study showed that small colony variants of *S. aureus* were detected at high number in diabetic foot infections (Hassan et al., 2016).

**Estimation of TNF-α in Diabetic Foot Patients**

The present data in table (5) revealed that the TNF-α mean serum level was-significantly-higher in diabetic foot patients (277.44 pg/mL) as compared with healthy control group (70.13 pg/mL) (P=0.0004). Similar findings were recorded by many studies (Archive et al., 2008; Xu et al., 2013; Yadav et al., 2017) that serum levels of TNF-α were significantly elevated in diabetic foot patients.

**Table 5 : Mean serum levels of TNF-α in diabetic patients-(II) with foot infections and healthy control groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>No</th>
<th>TNF-α (pg/mL) mean ±SE</th>
<th>Min.</th>
<th>Max.</th>
<th>P1 value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic patients</td>
<td>66</td>
<td>277.44</td>
<td>125</td>
<td>1000.000</td>
<td>0.0004</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>70.13</td>
<td>70</td>
<td>230</td>
<td></td>
</tr>
</tbody>
</table>
TNF-α were elevated in serum of diabetic patients with foot infections with peripheral diabetic-neuropathy. In TDM patients proinflammatory-cytokines acting vital role-in the autoimmune pathogenesis of β-cell destroying. Insulin resistance has been associated with abnormal secretion of proinflammatory cytokines like (IL-6) and TNF-α and decreased making of anti-inflammatory mediators like IL-4 and IL-10 (Xiao et al., 2014). A high mount of TNF-α, which suppress angiogenesis and cell reproduction in diabetic lesion and elevated apoptosis extents (Yadav et al., 2017).

Conclusion

Diabetic foot infections (DFIs) are important widely known health concerns. Learning pathogens associated with DFIs and their antimicrobial susceptibility patterns are useful to prepare guidelines for appropriate antimicrobial therapy. Our study showed that S. aureus was most common organism detected in diabetic foot ulcers. Imipenem was the most efficient antimicrobial agent. These data indicate that the TNF-α system is activated in diabetic foot patients.

Consent

All patients’ consents were taken before inclusion in the study.

Ethical Approval

Ethical Committee of the Babylon health directorate approved the study.

Acknowledgements

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