The Effect of Bacterial Infection on Oxidative Burst Activity in Leucocytes from Diabetic Patients

O. Famuyiwa, R. A. Sulimani, A. Tuwajiri, N. S. El-Sagir

Respiratory burst activity in phagocytes was assessed by measuring luminol dependent chemiluminescence (CL) in whole blood and isolated polymorphonucleocytes (PMNs) from four groups of subjects in the basal state and after stimulation with phorbol myristate acetate (PMA). The groups were: healthy controls (group I); diabetics with bacterial infection (group II); diabetics without infection (group III); and non-diabetics with bacterial infection (group IV). In the basal state with whole blood, there was a statistically significant difference in resting CL response only between groups I and II, (p<0.05, by ANOVA and t-test with Bonferoni’s adjustment). With isolated PMNs, there was no statistically significant difference between any of the groups. After stimulation with PMA, in whole blood there was a statistically significant difference, p<0.05 when group II was compared with groups I and III. With isolated PMNs, the only significant observation was between groups II and III, p<0.05. In the presence of bacterial infection, PMNs from diabetic patients demonstrated higher CL responses than controls and non-diabetic patients with infection. The findings suggest that PMNs from diabetic patients with bacterial infection can be functionally and metabolically activated. The susceptibility to infection in diabetic patients may not be due to impairment of the respiratory burst activity in PMNs.

Infections still account for significant morbidity and mortality in diabetic patients. In Saudi Arabia, among ambulant and hospitalized diabetic patients evaluated over a 5-year period, infections were the second most common complication, being present in 37.9% (Famuyiwa et al., unpublished data). This susceptibility to infection applies especially to patients who are poorly controlled. Several aspects of the host immune system have been investigated in order to determine the basis for this susceptibility. Polymorphonuclear cell (PMN) function in relation to chemotaxis,2-5 phagocytosis6-9 and intracellular killing ability8-11 have all been investigated with often conflicting findings.

Leucocyte chemiluminescence (CL) during phagocytosis was first described by Allen.12 It has
Table 1
Clinical characteristics and biochemical profile of the study subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>I Healthy controls</th>
<th>II Diabetics with infection</th>
<th>III Diabetics without infection</th>
<th>IV Non-diabetics with infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>15</td>
<td>31</td>
<td>10</td>
</tr>
<tr>
<td>Sex M/F</td>
<td>15/0</td>
<td>7/8</td>
<td>19/12</td>
<td>8/12</td>
</tr>
<tr>
<td>Age (years) range</td>
<td>15–55</td>
<td>21–70</td>
<td>14–70</td>
<td>20–70</td>
</tr>
<tr>
<td>mean</td>
<td>27</td>
<td>53</td>
<td>46.5</td>
<td>43.3</td>
</tr>
<tr>
<td>Type of diabetes IDD/MIDDM</td>
<td>NA</td>
<td>2/13</td>
<td>8/23</td>
<td>NA</td>
</tr>
<tr>
<td>Diabetes duration (years) range</td>
<td>NA</td>
<td>4–30</td>
<td>0.3–25</td>
<td>NA</td>
</tr>
<tr>
<td>mean</td>
<td></td>
<td>12</td>
<td>8.6</td>
<td>NA</td>
</tr>
<tr>
<td>Blood glucose (mmol/l) range</td>
<td>NA</td>
<td>8.9–18.2</td>
<td>6.8–14.9</td>
<td>NA</td>
</tr>
<tr>
<td>mean</td>
<td></td>
<td>13.4</td>
<td>11.3</td>
<td>NA</td>
</tr>
<tr>
<td>HbAl (%) range</td>
<td>NA</td>
<td>8.3–17.5</td>
<td>7.5–18.0</td>
<td>NA</td>
</tr>
<tr>
<td>mean</td>
<td></td>
<td>12.7</td>
<td>13</td>
<td>NA</td>
</tr>
<tr>
<td>Infection</td>
<td>UTI (3), sepsis (3), infected foot (3), TB (2), bronchietasis (1) dental abscess (1)</td>
<td>RIF/INH/ETH/PR / (2)</td>
<td>Pneumonia (5), pulm. TB (2), miliary TB (1), tuberculous abscess (1), UTI (1)</td>
<td></td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Gentamicin (5), ampicillin (3), cloxacillin (3), metronidazole (2), erythromycin (2), RIF/INH/ETH/PR / (2)</td>
<td>RIF/INH/ETH/PR / (4), ampicillin (2), erythromycin (2), ampicillin (1), ceftriaxone (1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NA: not applicable; UTI: urinary tract infection; TB: tuberculosis; Pulm: pulmonary; RIF/INH/ETH/PR: rifampicin + isoniazid + ethambutol + pyrazinamide.

( ): numbers of patients with infections and frequency of occurrence and use of antibiotics.

*Two patients with foot infections received more than one antibiotic.

subsequently been shown that CL is closely linked to bactericidal and opsonic activity in various types of phagocytic cells. Using the method of CL response and superoxide anion production, Shah et al. reported an impaired oxidative burst by leucocytes from diabetic patients. However, they studied only diabetic patients and controls without infection. Barbour et al. have reported that leucocyte CL response is significantly higher in subjects with infection when compared with controls without infection. They studied non-diabetic patients and they concluded that the leucocytes of the majority of subjects with active bacterial infection are in an activated state both functionally and metabolically.

In view of the conclusions of Shah et al., regarding impaired leucocyte function in diabetic patients without infection, we considered it important to study the leucocyte CL response in diabetic patients with infection to determine whether this impairment persisted or if there was activation as suggested by others.

**Materials and Methods**

The patients were recruited from those admitted to the King Khalid University Hospital, Riyadh. The study subjects comprised four groups as follows: group I—healthy controls (healthy blood donors), group II—diabetic patients with infection, group III—diabetic patients without infection, group IV—non-diabetic patients with infection. Among the diabetic patients with infection...

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(group II), 12 were taking insulin, two were taking oral hypoglycaemic tablets and one was following a prescribed diet only. Among those without infection (group III), 24 were taking insulin, four were taking oral tablets and three were following a prescribed diet. Characteristics of the study subjects are provided in Table 1.

Polymorphonuclear leucocytes isolation
Polymorphonuclear leucocytes (PMNs) were isolated according to the method previously described by Al-Tuwaigri et al. Briefly, blood was collected by venepuncture into sterile containers with heparin (10 IU/ml; Fischer Scientific Co., NJ, USA). Heparinized blood (10 ml) was mixed with 1 ml of Dextran T500 6% (w/v) in saline 0.9% (w/v) and kept at room temperature for 30–45 min after which the leucocyte-rich plasma layer was removed and layered over 3 ml of Nycodenz solution (Nyegaard and Co. AS, Torshov, Norway) in a 10 ml tube and centrifuged at 1800 rpm for 15 min to harvest the PMNs mixed with some erythrocytes. The erythrocytes were lysed by adding 5 ml of 0.87% ammonium chloride for 10 min on ice and centrifuged at 1700 rpm for 10 min. The supernatant was discarded and the sediment was suspended in 10 ml phosphate buffered saline (PBS) and centrifuged to remove the remaining ammonium chloride. The sediment was resuspended in PBS, counted and adjusted to the desired concentration. The viability of PMNs was assessed by 0.1% trypan blue exclusion and only PMNs suspensions with a viability of 90% or above were used.

Measurement of luminol-enhanced chemiluminescence
An LKB-Wallac 1251 luminometer with a constant temperature (37 °C) controller (Wallac Oy, 20101 Turku 10, Finland) connected to an Apple IIe computer was used. The reaction mixture consisted of 100 μl of PMNs suspension or whole blood and 900 μl medium containing 10 μm luminol (5-amino-2,3-dihydro, 1,4-phthalazinedione Sigma Chemical Corp., St Louis, MO, USA) and 2 ng/ml phorbol myristate acetate (PMA) Sigma Chemical Corp., St Louis, MO, USA. Light emission was recorded in millivolts (mV) and the readings were recorded at 2 min intervals for 30 min. CL emission was quantified as the peak height in mV.

![Graph](figure1.png)

Figure 1. Representative peak chemiluminescence responses after phorbol myristate acetate stimulation in study subjects. (a) Diabetics with infection; (b) Non-diabetics with infection; (c) Healthy controls; (d) Diabetics without infection.
### Table 2
Comparisons of peak chemiluminescence response in study subjects

<table>
<thead>
<tr>
<th></th>
<th>Whole blood</th>
<th>Polymorph leucocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>PMA stimulated</td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy controls</td>
<td>1.03 ± 0.1</td>
<td>18.61 ± 0.65</td>
</tr>
<tr>
<td></td>
<td>(12)</td>
<td>(12)</td>
</tr>
<tr>
<td>Group II</td>
<td>5.3 ± 2.8</td>
<td>92.12 ± 1.8</td>
</tr>
<tr>
<td>Diabetics with infection</td>
<td>(5)</td>
<td>(15)</td>
</tr>
<tr>
<td>Group III</td>
<td>8.46 ± 0.34</td>
<td>16.02 ± 2.3</td>
</tr>
<tr>
<td>Diabetics without infection</td>
<td>(10)</td>
<td>(31)</td>
</tr>
<tr>
<td>Group IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-diabetics with infection</td>
<td>2.29 ± 1.3</td>
<td>40.84 ± 23</td>
</tr>
<tr>
<td>ANOVA</td>
<td>f (3,25) = 2.95;</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>t-test*</td>
<td>p &lt; 0.05 between groups I and II only</td>
<td>p &lt; 0.05 between groups II and groups I, III only</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scheffe’s rule**</td>
<td>2.96</td>
<td>2.71</td>
</tr>
</tbody>
</table>

( ): number tested; PMA: phorbol myristate acetate.
ANOVA: Analysis of variance.
*Pair-wise comparisons with Bonferroni’s adjustment.
**Significant if value of calculated contrast is greater than indicated, inferences the same as with t-test.

### Statistics
An analysis of variance (ANOVA) was used to compare the peak CL response in the various groups and Student’s t-test with Bonferroni’s adjustment was used for pair-wise comparisons. The differences between group means were examined for significance by a comparison of the linear contrasts.

For two groups with means \( \bar{x} \), \( \bar{y} \), \( a_i = 1, a_j = -1 \) and all other \( a_k = 0 \), for the linear contrast \( L = \Sigma a_{ik} x_k \). The absolute value of the t-statistic, given as \( t = L/SE(L) \) where \( L \) is the standard error of the linear contrast is thus declared significant if it exceeds \( V - 1 \ F(0.05) \) using the Scheffe’s rule\(^2\) at the 5% probability level.

### Results
Table 1 provides the clinical parameters in the study subjects as well as relevant biochemical data in the diabetic patients. It also indicates the types of infection present in the subgroups of diabetic and non-diabetic patients as well as the types of antibiotics that were prescribed.

Table 2 summarizes the chemiluminescence (CL) data and Fig. 1 illustrates representative peak responses from four patients among the study subjects. Data for both whole blood and isolated PMNs are shown. Results with saline represent the unstimulated or basal CL. In the basal state with both whole blood and PMNs, luminol-dependent CL in the diabetic and non-diabetic subjects with or without infection, was higher than in healthy blood donors. The differences were statistically significant (p < 0.05) only in whole blood tests between the controls (group I) and diabetic patients with infection (group II). After stimulation with PMA, the peak CL responses were generally higher in patients with infection, diabetic (group II) and non-diabetic (group IV), than in the controls or diabetic patients without infection (group III) (Fig. 1). In whole blood tests, values in diabetic patients with infection were statistically significantly higher (p < 0.05) than results in healthy controls and diabetic patients without infection. In isolated PMN tests, there were no statistically significant differences in the baseline values for the groups (Table 1). After PMA stimulation, the only statistically significant comparison was between diabetic patients with infection (group II) and those without infection (group III), p < 0.05. Healthy controls had higher responses than diabetic patients without infection, but the difference was not statistically significant.

### Discussion
Infections remain a major problem in diabetic patients. Foot infections, urinary tract infection, pneumonia and mucocutaneous candidiasis are relatively common while malignant otitis externa and rhinocerebral mucormycosis occur almost exclusively in diabetic patients.\(^{21,22}\) There is still no agreement as to the mechanism underlying the susceptibility to infection in diabetic patients. Several limbs of the immune system have been investigated with conflicting results.\(^{2-11,22-25}\) More recently, excessive sorbitol production in leucocytes\(^{26}\) and glycosylation of proteins\(^{27}\) have been suggested as possible underlying pathogenetic mechanisms.

Shah \textit{et al.}\(^{18}\) studied superoxide anion production and CL response in leucocytes from diabetic patients and controls without infection. They observed decreased CL response after zymosan stimulation in the PMNs from diabetic patients compared with controls and they suggested that this might indicate impaired intracellular killing ability.
They concluded that this impaired CL response provided the basis for the susceptibility to infection in diabetic patients.

The present study included healthy controls, diabetic patients without infection as well as diabetic and non-diabetic patients with a variety of bacterial infections. Our findings in the diabetic patients without infection compared with healthy controls are similar to those of Shah et al. Our conclusion would have been the same as theirs if we had not studied patients with infection.

The CL response in the diabetic patients with infection was comparable with that in non-diabetic patients with infection and higher than in the healthy controls. Our results confirm observations previously made by Barbour et al. that in the presence of bacterial infection there is a marked increase in CL response of PMNs from non-diabetic patients. Furthermore, PMNs from diabetic patients have the capacity to respond in the same way.

CL response has been closely linked to bactericidal activity in various types of phagocytic cells. In addition, Barbour et al. suggested that the increased activity in PMNs during bacterial infection is an indication that they have been activated functionally and metabolically. The results from this study indicate that the phagocytic function of PMNs in diabetic patients with infection, as measured by CL activity, is not impaired. However, in the interpretation of these results, the possible confounding effects of some of the drugs that the patients were taking must be kept in mind. Various antibiotics have been shown to have a variety of effects on CL activity in polymorphs. Suppression was shown by some including amoxicillin, tetracyclin, trimethoprim-sulphamethoxazole, fusidic acid, rifampicin, isoniazid and stimulation by others such as ceftriaxone, enoxacin and norfloxacin. Only one of the non-diabetic patients received an antibiotic which potentially stimulates CL response while two diabetic and six non-diabetic patients received antibiotics that could inhibit the response. In addition, various bacterial pathogens have been shown to produce different CL responses in PMNs from healthy human subjects with some causing a rapid exponential rise and decay in CL while others produce a slow rise with a poorly defined peak.

These observations notwithstanding, it is unlikely that our findings are non-specific or artefactual. Our own preliminary studies in oncology patients with infection who were undergoing antimicrobial therapy showed severely impaired CL response (Famuyiwa et al., unpublished data). Our results have demonstrated adequate CL response by PMNs from diabetic patients with a wide variety of bacterial infections when compared with diabetics without infection, healthy non-infected controls and non-diabetic hospital patients with infection. They suggest that the susceptibility to infection in diabetic patients may not be caused by an impairment of the respiratory burst activity in PMNs. They also indicate the need for more careful studies to corroborate these findings while controlling for possible confounding factors.

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References


