**MATERIALS AND METHODS**

- The study population consisted of DM patients presenting for care of a diabetic ulcer located anywhere on the foot and non-DM patients (controls) with a leg ulcer. All the human study protocols were approved by the IRB at UCD and VA.
- Subject inclusion criteria: age 18 or older; ulcer size ≥2 to ≤25 cm²; ulcer duration of ≥4 weeks; no clinical signs of infection; glycosylated hemoglobin (HbA1c) <12%; and adequate circulation to the affected extremity.
- Debridement tissue was collected using sharp debridement technique and snap frozen for molecular analyses.
- Total RNA was isolated from tissues for RT-CPR assays. Total protein and nuclear extracts were isolated from wound tissues for Western blots, cytokine measurements, and NF-κB ELISA assays. Densitometry was performed on blots. Wound tissue lysates were used for TBARS assays.
- Statistic analysis was performed using Student’s t-test.

**RESULTS**

**Table 1: TLR pathway genes expressed in debridement wound tissue. Human diabetic wounds (n=8) show significantly higher mRNA/18s ratio compared to non-diabetic wounds (n=5) (P<0.05 vs. non-diabetic wounds)**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Non-diabetic wounds mRNA/18s ratio</th>
<th>Diabetic wounds mRNA/18s ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1</td>
<td>0.6 ± 0.1</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>TLR2</td>
<td>1.2 ± 0.3</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>TLR4</td>
<td>1.3 ± 0.2</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td>TLR6</td>
<td>0.2 ± 0.1</td>
<td>2 ± 0.5</td>
</tr>
<tr>
<td>MyD88</td>
<td>1.4 ± 0.2</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>IRAK-1</td>
<td>1.1 ± 0.1</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td>MD2</td>
<td>0.1 ± 0.04</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>NF-κB</td>
<td>0.8 ± 0.05</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1 ± 0.4</td>
<td>2.6 ± 0.6</td>
</tr>
</tbody>
</table>

**Figure 1: Western blot showing the MyD88 protein expression in non-diabetic and diabetic wound tissue (P<0.05 vs. non-diabetic).**

**Figure 2: The DNA-binding activity of nuclear NF-κB p65 in wound tissues was determined using ELISA technique (P<0.05 vs. non-diabetic).**

**Figure 3: IL-1β and TNF-α concentrations in wound tissues were determined by ELISA assay and express oxidative substances (TBARS) assay (P<0.05 vs. non-diabetic).**

**Figure 4: Lipid peroxidation in wound tissue lysates were determined using thiobarbituric acid reactive substances (TBARS) assay (P<0.05 vs. non-diabetic).**

**CONCLUSIONS**

- All the patients had DM for > 5 years (mean glucose of 132 ± 10 mg/dL and HbA1c of 7.5 ± 0.8%).
- TLR1, TLR2, TLR4, TLR6, MyD88, IRAK-1, NF-κB, IL-1β, and TNF-α mRNA expression were significantly increased compared to non-diabetic wounds (P<0.05) (Table 1). MyD88 and NF-κB expression were significantly increased in diabetic wounds vs non-diabetic wounds (P<0.05).
- Local IL-1β and TNF-α concentration were increased in diabetic wounds vs non-diabetic wounds (P<0.05).
- TBARS (surrogate oxidative stress marker) formation is elevated in diabetic wounds vs non-diabetic wounds (P<0.05).

**REFERENCES**


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