The Effect Of Typhoid Fever On Cytokines (Interleukin-6 And 8) And C–Reactive Protein Concentration

1. Department of clinical laboratory science, college of pharmacy / Baghdad University
2. Department of immunology, central laboratory health.

amalalhadithy49@yahoo.com

ABSTRACT

The activation of inflammatory cells, the release of their mediators and the excessive production of free radicals by typhoid fever may affect the circulating Interlukin–6, Interlukin–8, and C–Reactive Protein concentration. Sixty four patients with typhoid fever were recruited from the inflammatory bowel disease clinic of Kadhimiya Teaching Hospital (between June 2010 To November 2010). IL–6 and IL–8 were measured (by EASIA test) and C–reactive protein by (LATEX TEST KIT) in 64 typhoid fever patients and 30 healthy control groups. The study shows highly significant increase in the concentration of (IL–6, IL–8, and C–reactive protein) compared with the healthy persons respectively (P>0.0001).

Keywords: Typhoid fever, IL–6, IL–8, C–Reactive protein, Inflammation, Mediaters

1. Introduction

Salmonella typhi is a facultative intercellular pathogen that causes typhoid in humans. The bacterium enters the body orally after the intake of contaminated food or water, and upon reaching the small intestine, adheres to and invades the specialized M–cells and enterocytes. The pathogen is translocated to the intestinal submucosa and subsequently disseminates throughout reticuloendothelial system. S. typhi can be isolated from spleen, liver, bone marrow, and gall bladder during typhoid fever (Y0ung et al 2002). The host – pathogen interactions during infection with this bacterium remain incompletely characterized in the small intestine (Jones et al 1996). Salmonella species in general invade host cells or induce cellular cytotoxicity, the reasons for the host specificity exhibited by S. typhi and other salmonella serovars are not well understood. Unlike S. typhi and S. typhimurium cause enteritis in humans, which is characterized by self– limited fever and diarrhea and in some cases, dysentery. These symptoms are rarely observed during infection with S. typhi (Keusch 1991).
S. typhi is the causative agent of typhoid fever, a severe systemic infection in which neutrophils are scarce in intestinal infiltrates and leukocyte populations (Nguyen et al 2004). Infection of human colonic tissue explants with non typhoidal salmonella serotype, S.typhimurium results in expression of neutrophil chemo attractant interleukin (IL–8), but this response is not observed during infection with S. typhi (Raffatella et al 2005). These data illustrate that S. typhi possesses virulence mechanism that prevent the induction of host responses leading to neutrophil recruitment in the intestinal mucosa (Raffatella et al 2006). The initial interactions of salmonella serotype with the intestinal mucosa that lead to the production of IL–8 can be modeled using colonic epithelial cells (Eckmaan et al 1993 and McCormick et al 1993). Activation of basolaterally expressed toll like receptor by flagellin activates mitogen activated protein kinase pathways and IL-8 secretion in polarized cells (Gewirtz et al 2001, and Yu Y et al 2003). Only flagellated S. typhimurium can induce the release of IL–8 from polarized cell (Aldridge et al2003, Reed et al 2002 and Zeng et al 2003). The above observations suggest that virulence mechanism, which reduce IL-8 expression in colonic epithelial cells are expressed by S. typhi, but not by S. typhimurium (Parkhill et al 2001). An elevated IL–8 concentration does not allow any differential diagnostic conclusions to be reached what so over, instead, it is just an indicator of an ongoing inflammatory response of various causes (Rodriguez et al 2001). IL–8 can be produced by immune cells, mainly monocytes, macrophages, as well as by non immune cells such as endothelial or epithelial cells. Monocyte, macrophages secret IL–8 within a few hours (< 6 h) after coming into contact with bacteria, or bacterial toxins. Isolated high IL–8 concentration in conjugation with high IL–6 levels that persist over the course of several days, point to an activation of non immune cells, this may be a result of the interaction between bacteria or bacterial product (Thomas 1998). C–reactive protein was described as an acute phase reactant synthesized by hepatocytes in response to the pro inflammatory cytokine interleukin-6. The use of C–reactive protein as a biomarker becomes more common when a low cost, high sensitivity test for C–reactive protein was developed. C–reactive protein as well as the inflammatory cytokines, interleukin-6 and tumor necrosis factor (TNF) was noted to identify asymptomatic older subjects in the community who were at high risk of the future development of heart failure. C–reactive protein has been shown to exert direct adverse effect on the vascular endothelium by reducing nitric oxide release and increasing indothelin-1 production, as well as by inducing expression of endothelial adhesion molecules. These findings suggest that C–reactive protein may also play a causal role in vascular disease and could therefore be a target of therapy. However, elevated levels of C–reactive protein lack specificity: for example, acute and chronic infection, cigarette smoking, acute coronary syndromes, and active inflammatory states are frequently associated with elevated levels of C–reactive protein (Anker 2004, Castell et al 1990, Ridker 2001, Anand et al 2005, Vasan et al 2003, Venugopal et al 2005 and Mann et al 2004). Human interleukin-6 is a 184 amino acids polypeptide with potential O and N–glycosylation sites. It is produced by various cells, including T-and B-cells, monocytes, fibroblasts, keratenocytes, endothelial cells, mesangial cells, astrocytes, bone marrow stroma cells and several tumor cells. It regulates the growth and differentiation of various cell types with major activities on the immune system, hemapoiesis and inflammation. These multiple actions are integrated within a complex cytokine network, where several cytokines induce by IL–6 and the final effect result from
either synergistic or antagonistic activities between IL–6 and the other cytokines. IL–6 is also a major inducer of the acute phase reactions in response to inflammation or tissue injurt. IL–6 is also interacts with the neuroendocrine system, thus IL–6 is a pleiotropic cytokine with multiple endocrine, paracrine and possibly autocrine activities in various tissues (Gogos et al 2001, Moscovitz et al 1994, Sakamoto et al 1994, Kita et al 1994 and Lemoine et al 1994). The main objective for this work is to study the interaction of Salmonella typhi with human cytokines (IL–6, IL–8 and C-reactive protein).

2. Methodology

Subject and Methods: Sixty four patients with typhoid fever were recruited from the inflammatory bowel disease clinic of Kadhimiya Teaching Hospital. Diagnosis of the disease was based on standard clinical, histological features and chemical tests (Widal test) (Olopoenia et al 2004). The severity of the disease was evaluated by the sensitivity of abdominal pain, general well being extra intestinal manifestations of the disease and high fever. Thirty healthy persons served as control groups. Blood samples were collected, serum was separated immediately by low speed centrifugation and the serum was frozen at -20°C bending analysis. Method of Interleukin–6 analysis: Interleukin (IL–6) levels were measured by enzyme linked immunoassay. The Bio source IL–6–EASIA is a solid phase enzyme amplified sensitivity immunoassay performed on micro titer plate. The assay uses monoclonal antibodies (MAbs) directed against distinct epitopes of IL–6. Calibration and samples react with the capture monoclonal antibody (MAbs) coated on the micro titer well and with a monoclonal antibody (Mabs2) labeled with horse radish peroxidase (HRP). After an incubation period allowing the formation of a sandwich coated Mabs1–humanIL–6–Mabs2–HRP, the micro titer plate is washed to remove unbound enzyme labeled antibody. Bound enzyme–labeled antibody is measured through a chromogenic reaction. Chromogenic solution (TMB) is added and incubated. The reaction is stopped with the addition of stop solution and the micro titer plate is read at the appropriate wave length. The amount of substrate turnover is determined calorimetrically by measuring the absorbance which is proportional to the IL–6 concentration. A calibration curve is plotted and IL–6 concentration in the samples is determined by interpolation from the calibration curve (Gogos et al 2001). IL–8 the same as the method of IL–6. While the method for CRP (LATEX TEST KIT): Is for the qualitative and semi quantitative estimation of the C–Reactive Protein (CRP) in human serum samples. The assay is performed by testing a suspension of latex particles coated with anti-human CRP antibodies against unknown serum. The presence of visible agglutination indicates an increase in levels of CRP to a clinically significant level (Hagashi et al 1972).

3. Results & Discussion

To study the interaction of S. Typhi with human cytokines (IL–6 and IL–8) were analyzed by EALSA Test and C–reactive protein by Latex Titer kit The baseline characteristics of cases and control groups are provided in tables (1, 2, and 3) of the total subjects recruited. Interleukin–6 (IL–6) and Interleukin–8 (IL–8) were estimated in 64 cases and 30 controls.
show highly significant in the concentration of IL–8 and IL–6 compared with the healthy persons (table 1 and 2) respectively and (figure 1). (P >0.0001). Where C–Reactive Protein whose synthesis in the liver is regulated by the pro– inflammatory cytokine (IL–6) shows significant increase in serum concentration of patients with typhoid fever compared with the control groups (table 3, and figure 1). (P > 0.0001).

The vast majority of salmonella serotypes causes gastroenteritis in human, a few serotypes (known as Typhoidal Salmonella Serotypes) are human–adapted and cause typhoid fever. Gastroenteritis and typhoid fever differ dramatically with regard to symptoms and pathological changes, including the cellular composition of inflammatory infiltrates in the intestine (Santose et al 2001, and Zhang et al 2003). While gastroenteritis is characterized by intestinal infiltrates that are dominated by neutrophils, this cell type is scarce as cellular infiltrates of typhoid fever patients (Ponwollik et al 2004). In this study serum IL–8 concentration show highly significant increase (P>0.0001) compared with the control groups. Salmonella typhi has recently been implicated in preventing the generation of host responses (IL–8 production in the intestinal epithelial cells that lead to neutrophil influx (Rafatellu et al 2007, and Andrews et al 1995). These observations lead to reduce cytokine expression, neutrophil recruitment and fluid accumulation elicited by S.typhi (Stojilkovic et al 1995). These data suggest that S. typhi account for the scarcity of neutrophil in intestinal infiltrates of typhoid fever patients (Rajalingam et al 2005). The goal of this study was to determine by which the mechanism of IL–8 production in human intestinal epithelial cells. Previous reports demonstrate that salmonella serotypes elicited IL–8 secretion in T84 cells by producing flagellin (Virlogeux et al 1995, Ochiai et al 2008, and Kingsley and Baumler 2002). So flagellin secretion explains how S. typhi enables to produce IL–8 in epithelial cells (Bergman et al 2003).

Our data are the first suggestion that changes in the regulation of highly conserved genes (i.e. flagella biosynthesis) may be an important mechanism contributing to the evolution of human–adapted typhoidal salmonella serotypes. This study investigat the interaction of S. typhi with the human cytokines. The regulation of cell cycle is believed to be mediated through the MAP kinase pathway (Wang et al 2002). The aforementioned pathway also plays a crucial role in the production of inflammatory responses during infection with S. typhi (Hebbie et al 1997, and Sankovic et al 2002). Our results showed that the concentration of IL–8 is highly increased in patients with S. typhi (table 2, fig. 1). The cytokines IL–6 is important mediators for signs and symptoms of infectious diseases (Elias 1992, and Keru et al 1992). In patients with typhoid fever, the mean concentration of IL–6 in plasma was elevated (153.1437 pg/ml). Levels of the IL–6 were previously reported by Rodriguez-Gaspar et al,.(2001) to be significantly elevated in Chilean children with typhoid fever greater than the mean concentration in our patients. The reason for this disparity could be the fact that all our patients were adults, the lesser clinical severity of our cases, or differences in laboratory methodology. The mean concentration of plasma cytokine (IL–6) was significantly higher in our patients, which indicated predictive value for clinical outcome of treatment in typhoid fever (Yamashita et al 2003). CRP production is part of the non specific acute–phase response to most forms of inflammation, infection, and tissue damage and was therefore considered not to provide clinically useful information. Indeed, CRP values can never be diagnostic on their own and can only be interpreted at the bedside, in full knowledge of all
other clinical and pathological results. However, they can then contribute strongly to management, just as universal recording of the patients’ temperature an equally non specific parameter, which is of great clinical utility (Ridker et al 2003). Under various circumstances CRP may contribute to host defense against infection. This protein must have survival values. Microbial infection is a major driving force of change during evolution, and CRP has many features compatible with a role in innate immunity (Pasceri et al 2000). CRP has also been reported to stimulate tissue factor production by peripheral blood monocytes in vitro and could thereby have important pro-coagulant effects (Verma et al 2002). We have long speculated that CRP may have significant pro-inflammatory effects, and by binding to ligands exposed on cells or other autologous structures as a result of infections, inflammation, ischemia, and other pathologies by which triggering complement activation (Griselli et al 1999).

(Table 1): Concentration of IL6 in typhoid fever patients and control groups:

<table>
<thead>
<tr>
<th></th>
<th>IL 6</th>
<th>No.</th>
<th>minimum</th>
<th>maximum</th>
<th>Mean pg/ml</th>
<th>Std. dev.</th>
<th>Std. error</th>
<th>Degree of freedom</th>
<th>Sig (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>15</td>
<td>21.9</td>
<td>66.9</td>
<td>39.3</td>
<td>13.7</td>
<td>3.5</td>
<td></td>
<td>47</td>
<td>0.001</td>
</tr>
<tr>
<td>patients</td>
<td>34</td>
<td>121.9</td>
<td>377.3</td>
<td>153.1</td>
<td>49.8</td>
<td>8.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Table 2): Concentration of IL8 in typhoid fever patients and control groups.

<table>
<thead>
<tr>
<th></th>
<th>IL 8</th>
<th>No.</th>
<th>minimum</th>
<th>maximum</th>
<th>Mean pg/ml</th>
<th>Std. dev.</th>
<th>Std. error</th>
<th>Degree of freedom</th>
<th>Sig (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>30</td>
<td>12.4</td>
<td>59.2</td>
<td>34.0</td>
<td>10.7</td>
<td>2.00</td>
<td></td>
<td>92</td>
<td>0.001</td>
</tr>
<tr>
<td>patients</td>
<td>64</td>
<td>24.3</td>
<td>923.5</td>
<td>131.6</td>
<td>116.5</td>
<td>14.5</td>
<td></td>
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</tr>
</tbody>
</table>

(Table 3): Concentration of CRP in typhoid fever patients and control groups.

<table>
<thead>
<tr>
<th></th>
<th>CRP</th>
<th>No.</th>
<th>minimum</th>
<th>maximum</th>
<th>Mean</th>
<th>Std. dev.</th>
<th>Std. error</th>
<th>Degree of freedom</th>
<th>Sig (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>10</td>
<td>6</td>
<td>12</td>
<td>8.8</td>
<td>3.2</td>
<td>1.6</td>
<td></td>
<td>28</td>
<td>0.001</td>
</tr>
<tr>
<td>patients</td>
<td>20</td>
<td>12</td>
<td>96</td>
<td>37.2</td>
<td>28.8</td>
<td>6.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(Figure 1) The concentration of IL6, IL8, and CRP in typhoid fever patients and control groups (P > 0.001)

4. Conclusion

Typhoid fever patients showed highly significant increase in the concentration of IL–6, IL–8, and CRP which may be of clinical significant.

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