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## Estimation of Interleukin-8 Level in Diarrheic Children Infected with Rotavirus

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### ABSTRACT

**Background and Objectives:** Rotavirus is the most important cause of severe diarrhea in children worldwide. We have investigated that certain cytokines especially interleukin- 8 (IL-8) as a chemokine, may play an important role in the pathogenesis of and the protection against rotavirus disease in children. The purpose of this study was to reveal the association between IL-8 and child diarrhea with rotavirus.

**Methods:** Stool samples were collected from 150 children under 5 years of age who developed diarrhea and 45 from non-diarrheic children of equivalent age. All samples were detected for rotavirus and serum IL-8 level. The study was undertaken from May to December of 2006. Enzyme linked immunosorbant assay (ELISA) was applied for detection rotavirus and IL-8.

**Results:** Among the children, female patients with breast feeding were less infected with rotavirus compared to males with artificial and mixed feeding. The IL-8 concentration was highly significantly between the study groups ( $P < 0.01$ ). Regarding the gender, serum IL-8 level was significantly higher in males than females ( $P < 0.05$ ). However, no significance prevalence in IL-8 level was recorded between gender in healthy control group ( $P > 0.05$ ).

**Conclusions:** Rotavirus infection induces the expression of chemokines as IL-8. These data support the hypothesis that chemokine secretion may play a role in the initiation and modulation of the immune response to rotavirus infection.

**Key words:** Rotavirus, Diarrhea, Children.

### INTRODUCTION:

Cytokines comprise a diverse group of small proteins with both pro- and anti-inflammatory properties and are increasingly recognized to play critical roles in the pathogenesis and immunity of infectious diseases<sup>1</sup>. However, chemokines constitute a superfamily of 8- to 10-kDa inducible secreted pro-inflammatory cytokines that serve as chemoattractants and cell activators in immune and inflammatory responses<sup>2</sup>. It is now recognized that chemokines are elements of a complex cell-signaling network that regulates kinesis, activation, and proliferation of a large number of cells, especially those with important roles in Ag-specific immunity<sup>2</sup>. Interleukin-8 is a

cated in pathogenesis and immunity in several diseases<sup>3</sup>. It is a chemotactic and cell-activating cytokine that is synthesized by epithelial cells and induced in response to enteric pathogens<sup>4</sup>. Rotavirus inoculation increased IL-8 mRNA levels in cultured intestinal epithelial cells within 2 hrs of infection. The IL-8 gene promoter region includes binding sites for transcription factors<sup>5,6</sup>. Rotavirus is a genus of double-stranded RNA virus in the family Reoviridae. It is the leading cause of severe diarrhea among infants and young children<sup>7</sup>. By the age of five, nearly every child in the world has been infected with rotavirus at least once<sup>8</sup>. However, with each infection, immunity develops and subsequent infections are less severe<sup>9</sup>. In developing countries, around 611,000

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of age die from rotavirus infection each year<sup>10</sup> and almost two million more become severely ill<sup>11</sup>. Rotavirus in the gut, replicate at the site of entry and cause disease by local tissue damage<sup>12</sup>. In such mucosal infections, systemic memory cells are frequently unable to prevent clinical symptoms, optimal protective immunity correlates with the presence of effectors cells or local antibody at mucosal sites<sup>12,13</sup>. These data support the hypothesis that IL-8 secretion may play a role in the initiation and modulation of the immune response to rotavirus infection. The aim of the present study was estimation of IL-8 level in diarrheic children infected with rotavirus.

#### **MATERIALS AND METHODS:**

The study was carried out in Al-Mansour Pediatric Hospital, Baghdad/Iraq from May to December 2006. The study population included 150 children under 5 years of age hospitalized for diarrhea consider as a patient group and 45 controls of the same age and sex admitted for causes other than acute gastrointestinal disease to serve as a control group. All subjects were selected by simple random sampling. Rotavirus antigen in stool samples was detected with a commercial ELISA kit (Rotavirus ELISA kit, DAKO-patts, Denmark). Both groups were studied for IL-8 concentration detection using commercial ELISA kit. Interleukin -8 levels was measured using the commercially available human IL-8 (ELISA) Kit for the quantitative determination. The human IL-8 Kit measures human IL-8 level by sandwich ELISA. The assay uses two monoclonal antibodies against two different epitopes of human IL-8. For evaluation IL-8 level, a calibration curve is constructed by plotting the absorbance values against the corresponding IL-8 concentration. Analysis of data was performed by using statistical package for social science (SPSS) version 11.5. Results are expressed as mean  $\pm$  S.E. Differences in proportions were assessed by a chi-square test. In cases in which the expected value for a cell was

less than 5, Fisher exact test was used. Statistical differences were determined by t-test when compared between two groups. P value  $< 0.05$  was considered statistically significant.

#### **RESULT:**

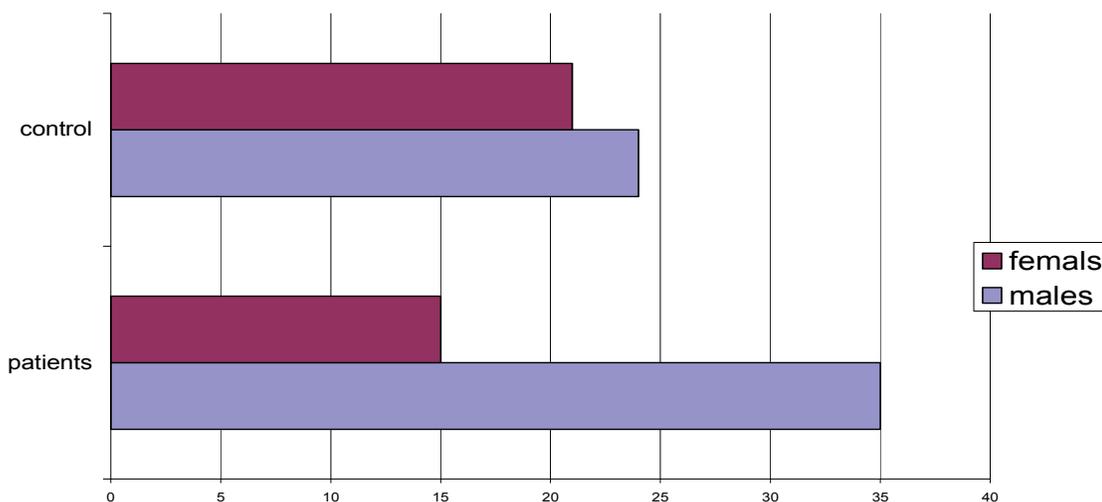
Of the 150 samples from the diarrhea group children and 45 samples from healthy control children, 50 (41.6 %) and 0 (0%) were positive for rotavirus respectively. The virus detection prevalence was highly significantly between the two study groups ( $P < 0.0001$ ). (Table 1) presents study groups according to their ages, there was no significant differences in age between the groups ( $p > 0.05$ ).

Regarding gender distribution, females were more infected with rotavirus compared to males (Figure 1). Patients with breast feeding were less frequently infected with rotavirus than artificial and mixed feeding (Figure 2). T-test revealed high significant differences in IL-8 level (mean  $\pm$  SE) among patient and healthy control groups ( $p < 0.01$ ) (Table 2).

Measuring IL-8 levels by T-test in both sexes, we found significant difference in IL-8 level between females and males in serum of patient group ( $p < 0.05$ ). However, inverse result was found between males and females in healthy control group ( $p > 0.05$ ) (Table 3).

**Table 1:** Age distribution of positive rotavirus in patients and healthy control.

Study Groups	Age in years							P Chi-test
	No.	No. of positive rotavirus	%	Mean	Min	Max	SD	
Patients	150	50	41.6	2.4000	1.00	5.00	1.27775	P>0.05
Healthy control	45	0	0	2.6889	1.00	5.00	1.39516	

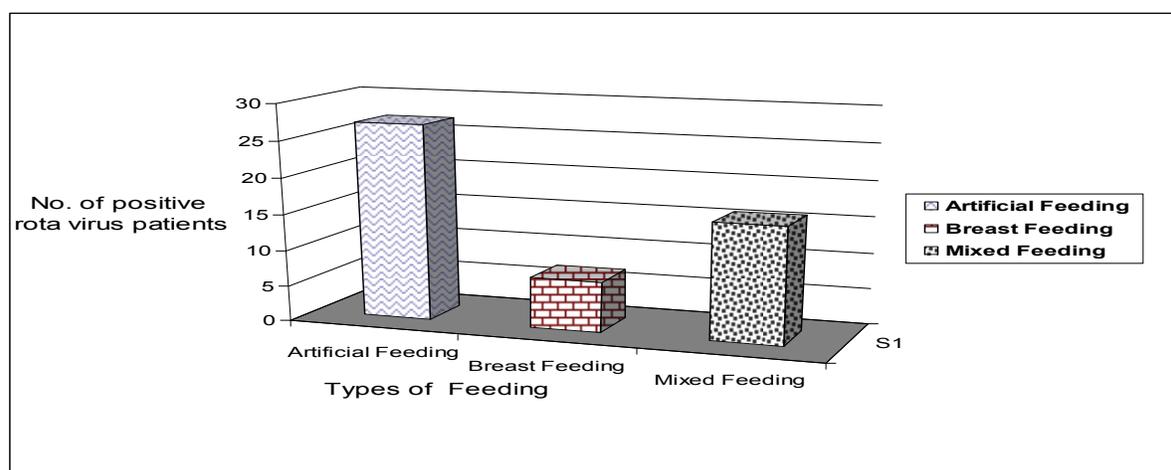


**Figure(1):**Gender distribution of rota virus positive patients and rotavirus negative healthy control

**Table 2:** The difference in mean serum IL-8(pg/ml) between study groups.

Study Groups	No.	IL-8 level	P (T test)
		Mean ± SE	
Patients	50	8860.0±920.48	P < 0.01
Healthy control	45	829.0±58.614	

Highly significant P < 0.01; IL-8: Interleukin-8; P: Probability



**Figure 2:** Distribution of rotavirus positive patients according to types of feeding.

**Table 3:** Gender distribution of mean serum IL-8(pg/ml) in the study groups.

Study Groups	Gender	No.	IL-8 level	P (T test)
			Mean ± SE	
Patients	M	31	10520±1163	P < 0.05
	F	19	6150±1316	
Healthy control	M	27	861±72.9	NS
	F	18	776±99.7	

**DISCUSSION:**

The number of children with positive rotavirus in this study included 50 cases (41.6 %). Our result supports the previous results that rotavirus is responsible for 20-60 percent of pediatric admissions to hospitals because of gastroenteritis in developed as well as in developing countries<sup>14,15</sup>. Indeed, in Erbil City the percentage of rotavirus was 37%<sup>16</sup>. However in another study rotavirus was detected in (66.2%)<sup>17</sup>. Males have been noted to be at higher risk for infection than females but Chi-Square test revealed no significant differences between them ( $P > 0.05$ ). Similar findings were reported by Rheingans *et al.*, and Trung *et al.*,<sup>18,19</sup>. Our data showed during the study that infant who were on artificial feeding have higher prevalence rate of rotavirus (54 %) than infant who were on natural feeding rate (14 %) and mixed feeding (32 %) this result agreed with the results of other investigators who found that Bottle-fed children and those cared at child care centers showed higher relative frequency of rotavirus gastroenteritis<sup>14,20</sup>. Breastfed infants develop a probiotic-rich gut microflora with less pathogenic bacteria, compared with formula-fed individuals<sup>21</sup>. This effect has been considered one of the mechanisms that decrease the rate of infectious diarrhea in breastfed infants<sup>22</sup>. It has been demonstrated that human milk is a source of lactic acid bacteria for the infant gut<sup>23</sup>. The induction of IL-8 expression by rotavirus is a well-established phenomenon<sup>24</sup>. Our data showed in a quantitative assay, the extent of IL-8 induction by rotavirus in diarrheic children. T-test revealed high significant differences in IL-8 level (mean  $\pm$  SE) among study groups, (rotavirus positive diarrheic patient group and rotavirus negative healthy control group ( $p < 0.01$ )). By measuring IL-8 levels by T-test in both sexes, we found significant difference in IL-8 level between females and males in serum of patient group ( $P < 0.05$ ). However, no significant differences were found between males and

group ( $p > 0.05$ ). The increase concentration of IL-8 in our results is consistent with previous studies in which IL-8 protein secretion increased in rotavirus infected cells<sup>24,25</sup>. Jiang *et al.* reported that the level of IL-8 was elevated not significantly in the sera of patients with rotavirus diarrhea<sup>26</sup>. However, Azim *et al.* compared many cytokines as TNF- $\alpha$ , IFN- $\gamma$ , IL-8, and IL-10 in children with rotavirus diarrhea than in the healthy control children, they found that IFN- $\gamma$  was the only cytokine that was significantly higher<sup>27</sup>. The decrease of IL-8 concentration in control group of our study is agreed with the result of Brasier *et al.* who stated that IL-8 synthesis, low or undetectable in normal noninflamed tissue<sup>28</sup>. Casola *et al.*<sup>29</sup> in their study reported that the increase concentration of IL-8 may be due to the IL-8 gene promoter. Results of Holtmann *et al.* stated that IL-8 gene expression requires at least three different signal transduction pathways<sup>30</sup>. The importance of IL-8 during rotavirus infection, have been shown to promote cell proliferation and epithelial repair<sup>31, 32</sup>. These data suggest that cytokine IL-8 may be an important mediator of the host response to viral gastroenteritis pathogens

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such as rotavirus.

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