Evaluation of C-Reactive Protein in Iraqi Children Presented with Acute Enteropathogenic Escherichia Coli Associated Diarrhea with Special Emphasis to Age and Gender

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ABSTRACT

Background and Objectives: The aim is to determine the role of Enteropathogenic Escherichia coli (EPEC) serotypes (O111, O55, O26, O86, O119, O127, O114, and O142) in C-reactive protein (CRP) level in acute diarrhea among children; to determine the possible effect of age and gender in CRP level.

Methods: Culture of stool samples from 64 children in MacConkey agar for primary isolation of E.coli and confirmed via API20E. Sero-grouping of E.coli performed via specific antisera. CRP detected by latex agglutination.

Results: In this study, 64 children with diarrhea were involved. The mean age was 2.92 years. Males represent 34/64, 53.1%. The range of CRP level 12-96 mg/dl. EPEC1 and EPEC3 not detected. EPEC2 detected among 14/64, 21.9% and EPEC4 in 18.8%. Among the age group <1 year, EPEC2 detected in 9.38%, with mean CRP level 12mg/dl, 3.13%, 48 mg/dl 6.25%. EPEC4 detected in 2/64, 3.13%, with mean CRP level 12mg/dl. Age group 1-4 year, EPEC2 detected in 6/64, 9.38%, with mean CRP level 24mg/dl. EPEC4 detected in 6/64, 9.38%; with mean CRP level 12mg/dl, 24mg/dl, 48 mg/dl 3.13% in each. Among the age group 4.1-8 year, EPEC4 detected in 4/64, 6.25%, with CRP mean level 48 mg/dl. EPEC not detected in 8-10 year age group. EPEC2 detected in male 28/64, 43.75% and the CRP level range from 12-96mg/dl. EPEC2 detected in 22/64 females, 34.38%; CRP level range from 12-96mg/dl. No significant difference or correlation between gender and CRP level detected. EPEC4 detected in males 26/64, 40.63%, CRP level range from 12-96mg/dl. EPEC4 detected in 26/64 females, 40.63%, CRP level range from 12-96mg/dl. No significant difference or correlation between gender and CRP level was detected. EPEC2 and CRP level significantly correlated with patients age. while infection with EPEC2 inversely correlated EPEC4. No significant correlation between gender and EPEC serotypes.

Conclusion: Although EPEC2 and EPEC4 frequently detected in diarrhea cases of 1-8 years old, only EPEC2 positively correlated with age. Even with a fluctuation in CRP level, it was positively correlated with age and not correlated with EPEC serotypes and gender.

Key Words: EPEC, age, gender, CRP

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ÖZET

Amaç: Amaç, çocuklarda akut diyarede C-reaktif protein (CRP) seviyesinde Enteropathogen Escherichia coli (EPEC) serotiplerin (O111, O55, O26, O86, O119, O127, O114 ve O142) rolünü; CRP düzeyine yaş ve cinsiyetin olası etkisini saptamaktır.

Yöntem: MacConkey agarında 64 çocuk E.coli'nin primer izolasyonu için dışkı örneklerinin kültürü yapılmış ve API20E ile doğrulanmıştır. Belirli antiserumlarla E.coli sero grupları yapılmıştır. Lateks aglütinasyonu ile CRP saptanmıştır.

Bulgular: Bu çalışmaya 64 diyareli olan çocuklar dahil edildi. Ortalama yaş 2.92 yıl idi. Erkekler 34/64, %53.1’i temsil ediyordu. CRP seviyesi 12-96mg/dl aralığında saptandı. EPEC1 ve EPEC3 saptanmadı. EPEC2 14/64, %21.9 oranında ve EPEC4 ise %18.8 olarak tespit edildi. <1 yıl yaş grubunda EPEC2 %9.38 oranında saptandı; % 3.13’ünde ortalama CRP seviyesi 12mg/dl; % 6.25’inde ise 48 mg/dl olarak saptanmıştır. EPEC4 2/64, %3.13 oranında, ortalama CRP seviyesi 12mg/dl tespit edildi. Yaş grubu 1-4 yıl arasında, EPEC2 6/64, %9.38, ortalama CRP seviyesi 48mg/dl tespit edildi. EPEC4, 6/64, %9.38; ortalama CRP seviyesi 12mg/dl, 24mg/dl, 48mg/dl olmak üzere herbirinde %6.25 olarak ortaya çıktı. 4.1-8 yaş grubunda, EPEC4 4/64, %6.25, CRP ortalaması 48mg/dl tespit edildi. 8 yaş üzeri grupta EPEC4 saptanmamış. 28/64 erkekte EPEC2 tespit edildi (%43.75) ve CRP seviyesi 12-96mg/dl aralığında tespit edildi. 22/64 kız çocuklarında EPEC2 tespit edildi (%34.8). Ortalama CRP seviyesi 12-96mg/dl aralığında tespit edildi. EPEC4, 26/64 kız çocukta (%40.63), ortalama CRP seviyesi 12-96mg/dl aralığında tespit edildi. EPEC4 ve CRP düzeyi arasında anlamlı bir fark veya korelasyon saptanmadı. EPEC2 ve CRP düzeyi arasındaki ilişki de doyurucu korelasyonu göstermiyor, cinsiyet ve EPEC serotipleri arasında anlamalı bir ilişki yoktur.

Sonuç: Her ne kadar 1-8 yaş arası diyare vakalarında EPEC2 ve EPEC4 sıklıkla tespit edilmiş olsa da, sadece EPEC2 yaşa pozitif olarak ilişkilidir.

Anahtar Sözcükler: EPEC, yaş, cinsiyet, CRP

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INTRODUCTION

Diarrhea is usually defined as the passage of loose or watery stools, usually at least three times in 24 hours and the importance is put onto the change in stool consistency rather than frequency, and the usefulness of parental insight in deciding whether children have diarrhea or not (1).

The diarrheagenic E.coli pathotypes that cause diarrhea include EPEC; enterotoxigenic E.coli; ETEC; enteroinvasive E.coli; EIEC; Shiga toxin-producing E.coli, STEC, diffusely adherent E. coli, DAEC and enteraggregative E. coli, EAEC. These pathotypes are defined by the presence or absence of one or more definable E. coli virulence factors (2). EPEC strains are diarrheagenic E. coli, which usually are classified by a combination of oligosaccharides (O), flagella (H), and capsular (K) antigens. They are associated with outbreaks of infantile diarrhea among children in developing countries. In contrast to the limited importance of EPEC in industrialized countries, EPEC is a major cause of diarrhea in developing countries(3). EPEC strains continue to cause severe and sometimes fatal infantile diarrhea, particularly in Africa. Increased efforts at diagnosis, defining the clinical spectrum of disease, understanding pathogenic mechanisms, and delineating immune responses are desperately needed to develop new strategies to combat EPEC(4).

CRP is ring shaped, pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells and some types of bacteria in order to activate the complement system via the C1q complex(5). It enhances phagocytosis by macrophages via opsonin-mediated phagocytosis, which express a receptor for CRP. It plays a role in innate immunity as an early defense system against infections. CRP rises within 2-6 hours of the onset of inflammation, up to a 50,000-fold, and peaks at 48 hours(6). Its half-life of 18-19 hours is constant, and therefore its level is determined by the rate of synthesis in the and production from liver as well as by other cells such as adipocytes and hence the severity of the precipitating cause(7). Normal concentration of CRP in healthy human serum is between 5 and 10 mg/L, increasing with aging(8).

The aim of the present study is to determine the possible correlation between EPEC serotypes (O111, O55, O26, O86, O119, O127, O114, and O142) and CRP level in acute diarrhea among children. Also to determine the possible effect of age and gender in CRP level.

MATERIALS and METHODS

A total of (64) diarrhoeic child attended to outpatient’s clinic of Baqubah teaching hospital during a period from January 2015 to December 2015 enrolled in this study. This study conducted according to the principles of Helsinki declaration. Dully filled consent obtained from all patient’s parents before participating in the study. Approval of ethical review Committee (Issue No. 1/232-15, January 2015), College of medicine –Diyala University-Iraq taken prior to initiation of the work.

Isolation and identification of E.coli

Gram staining technique applied for microscopic identification of gram-negative bacilli. Stool culture was done using MacConkey agar for determination of E.coli according to pinky colony morphology due to lactose fermentation (9).

E.coli typing

API 20E system from bio Merieux –France used for biochemical characterization of E.coli according to manufacturer instructions (10). E.coli typed by direct slide agglutination technique in to four types using specific trivalent antisera from Bio-Rad-France. Antibodies specific for O111,O55 ,O26 antigens for E.coli type 1 ;antibodies specific for O86,O119,O127 antigens for E.coli type 2 ;antibodies specific for O125,O126,O128 antigens for E.coli type 3 ; antibodies specific for O114,O124,O142 antigens for E.coli type 4(11).

Procedure

Physiological saline used as a negative control. If the negative control is positive (agglutinates), the strain is auto agglutinating, i.e. O rough. Slide agglutination with O antisera according to statens serum institute procedure(12).

The E.coli grown over night on a suitable agar medium not inhibiting motility. A small drop of antiserum 20 µl on a glass slide was applied. Culture from a single colony to each drop of antiserum was transferred via toothpick and mixed well and give milky turbidity. Titled the slide for 5 - 10 seconds. The reaction read with naked eye by holding the slide in front of a light source against a black background (indirect illumination). A positive reaction is seen as a visible agglutination. A negative reaction is persistence of the homogenous milky turbidity. A late or weak agglutination considered negative.

CRP-latex agglutination

Reagents and samples allowed reaching room temperature. About 50 µL of the sample and one drop of each positive and negative controls were added into separate circles on the slide test. Then the CRP-latex reagent was mixed vigorously before using and 50 µL was added next to the samples to be tested. The drops mixed with a stirrer, spreading them over the entire surface of the circle. The slide was placed on a mechanical rotator at 80-100 r.p.m. for 2 minutes. False positive results could appear if the test read later than two minutes. The approximate CRP concentration in the patient sample is calculated as follow(13) : 6 x CRP Titer = mg/L. The normal value of CRP in children was 0-10 mg/L (14).

Statistical analysis

Spearman’s test (rho) for categorical and non categorical data used for correlation. The level of significance was 0.05 to 0.01 (two-tail). The level of confidence limits was 0.05.Statistical analysis performed using SPSS for windows version 15 (15, 16).

RESULTS

In this study, (64) children with diarrhea were involved. As shown in table 1 the minimum age of infected children was 4 months while maximum age was 10 years and the mean age was 2.92 years. Males represent 34/64, 53.1% and the rest 30/64, 46.9% were females. The minimum CRP level 12mg/dl while maximum was 96mg/dl . As shown in table 2 , EPEC1 and EPEC3 not detected in all patients. EPEC2 detected among 14/64, 21.9% and EPEC4 in 12/64 ,18.8%. Table (3) shown that among the age group <1 year, EPEC2 detected in 6/64 , 9.38%, with CRP level 12mg/dl 3.13%, 48 mg/dl 6.25%. EPEC4 detected in 2/64 , 3.13%, with CRP level 12mg/dl 3.13%. Age group 1-4 year, EPEC2 detected in 26/64 , 40.63%, with CRP level 24mg/dl, 9.38% . EPEC4 detected in 6/64, 9.38% , with CRP level 12mg/dl, 24mg/dl, 48mg/dl 3.13%. Among the age group 4.1-8 year, EPEC4 detected in 4/64 , 6.25% , with CRP level 48 mg/dl. EPEC not detected in >8 years age group.

As shown in table 4 , EPEC2 detected in 28/64 male, 43.75% and the CRP level range from 12-96mg/dl . EPEC2 detected in 22/64 females, 34.38% , CRP level range from 12-96mg/dl . No significant difference or correlation between gender and CRP level was detected. EPEC4 detected in 26/64 males, 40.63%, CRP level range from 12-96mg/dl . EPEC4 detected in 26/64 females 40.63%, CRP level range from 12-96mg/dl . No significant difference or correlation between gender and CRP level was detected. As shown in table 5 infection with EPEC2 and CRP level significantly correlated with patients age. Infection with EPEC2 inversely correlated EPEC4 . Infection with any EPEC serotypes was not correlated with patient gender.
Table 1: General Description of Patients’ Age, Gender And CRP Level

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<th>Parameters</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean±SD</th>
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<tbody>
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<td>Age</td>
<td>Minimum</td>
<td>4(months)</td>
<td>10 years</td>
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<tr>
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<td>Maximum</td>
<td>2.92 ± 2.16(years)</td>
<td>12</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>34(53.1%)</td>
<td>30(46.9%)</td>
</tr>
<tr>
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<td>Female</td>
<td>30(46.9%)</td>
<td>28(42.2%)</td>
</tr>
<tr>
<td>CRP(mg/dl)</td>
<td>Minimum</td>
<td>12</td>
<td>96</td>
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<tr>
<td></td>
<td>Maximum</td>
<td>46.875 ± 33.71943</td>
<td>64(100%)</td>
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Table 2: Serotypes of EPEC In Diarrheic Children

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<th>Parameters</th>
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Table 3: EPEC Distribution According To Age And CRP Level In Diarrheic Children

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<tr>
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<td>2(3.13%)</td>
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<td>Negative</td>
<td>10(15.62%)</td>
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<tr>
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<td>EPEC3</td>
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Table 4. Distribution of *EPEC* According to Gender And CRP Level In Diarrheic Children

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</tr>
<tr>
<td></td>
<td>Negative</td>
<td>6(9.38%)</td>
<td>6(9.38%)</td>
<td>6(9.38%)</td>
<td>10(15.62%)</td>
<td>26(40.63%)</td>
<td>0.654</td>
<td>0.884</td>
<td>0.092</td>
<td>0.607</td>
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<tr>
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<td>Positive</td>
<td>8(12.5%)</td>
<td>8(12.5%)</td>
<td>6(9.38%)</td>
<td>12(18.75%)</td>
<td>34(53.13%)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Female</td>
<td>EPEC4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>4(6.25%)</td>
<td>9.231</td>
<td>0.026</td>
</tr>
<tr>
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<td>Positive</td>
<td>10(15.62%)</td>
<td>4(6.25%)</td>
<td>6(9.38%)</td>
<td>6(9.38%)</td>
<td>26(40.63%)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>10(15.62%)</td>
<td>4(6.25%)</td>
<td>10(15.62%)</td>
<td>6(9.38%)</td>
<td>30(46.87%)</td>
<td>ND</td>
<td>ND</td>
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ND: Not detected
In current study, EPEC4 detected among 40.63% of males and females. CRP level range 12-96mg/dl. Flocculation of CRP levels reflects the low grade inflammatory response for noninvasive EPEC. No significant correlation between gender and CRP level was detected. This may attributed to the limited or even absent role of hormones at this stage of life.EPEC2 and CRP level significantly correlated with patients age and this come in line with others (31). EPEC4 inversely correlated with EPEC4 and this may attributed to the competition on binding receptors that expressed on intestinal mucosa.

No significant correlation between gender and EPEC serotypes reflects the importance of predisposing factors and availability of receptors for attachment and colonization regardless of patients’ gender.

CONCLUSION

Although EPEC2 and EPEC4 frequently detected among diarrhea cases of 1-8 years old, only EPEC2 positively correlated with age. Even with a fluctuation in CRP level, it was positively correlated with age and not correlated with EPEC serotypes and gender.

Conflict of interest

No conflict of interest was declared by the authors.

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