Determination of Interleukin-8 (IL-8) Levels In Pneumonia Patients

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Abstract

The result of interleukin-8 level through acute and chronic phases in pneumonia patients sera was showed high level in patients compared with healthy persons (0.194 ± 0.046, 0.095 ± 0.006 pg / ml , respectively) with a significant difference statistically (P 0.05). The results of interleukin 8 levels in the age group (2 months -3 years) higher than the age groups (age 15-60years) (age 60-85years) (0.154 ± 0.04, 0.131 ± 0.02, 0.113 ± 0.2 pg / ml , respectively) with no significant difference (P   005).

Keywords: Interleukin-8(IL-8), Pneumonia disease

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تحديد مستوى الانترليوكين 8–في المرضى المصابين بذات الرئه

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الخلاصة:

ظهرت نتائج قياس الانترليوكين 8 خلال الطورين الحاد والمزمن في مصل مرضى ذات الرئه كانت باعلى المستويات مقارنه بالاصحاء (0.194 ± 0.046 , 0.095 ±0.006 بيكو غرام /مل على التوالي) مع فرق معنوي احصائيا (P<0.05).كذلك في الفئه العمريه (شهرين–3سنوات) اظهرت نتائج قياس انترليوكين 8 اعلى المستويات مقارنه مع بقيه الاعمار (15–60 سنه) (60–85 سنه) (0.154± 0.04, 0.131 ± 0.02, 0.113±0.2 بيكو غرام /مل على التوالي)مع بفرق غير معنوي P<0.05.

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Introduction

Pneumonia is the leading cause of mortality among children under five years of age [1].Bacterial pneumonia is leading to infect all ages while aspiration pneumonia infect infants and children with age less than 5 years, *Mycoplasma pneumonia* infect young adult [2]. Asserts that occurrence of pneumonia is often experienced in early childhood than at any other age. Lack of rapid, commercially available and accurate laboratory tests for most pathogens makes it very difficult to identify the cause of childhood pneumonia. Children had previously been excluded from treatment guidelines of differences between adults and children in frequency and type of underlying illnesses and causative agents [3]. Disease development result from disorder in draining function of bronchi or phagocytic activity of white blood cell or alveolar macrophage or presence of Immunodeficiency result from infection with other diseases [4]. Pneumonia is one of the most common nosocomial infections occurring in hospitalized patients. Hospital-acquired pneumonia (HAP) is pneumonia that occurs more than 48 hour after admission[5]. Interleukin-8 (IL-8), also known as GCP-1, NAP-1, and CXCL8, is a

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heparin-binding, (8-9) kDa member of the alpha, or CXC family of chemokines. Currently, there are 15 human CXC family members that generally range from (8-12) kDa in size. The majority are found on human chromosome, all contain a typical three β-sheet/one α-helix structure, and most show an N-terminal GluLeuArg tripeptide motif [6]. Human IL-8 is synthesized as a 99 amino acid precursor that contains 20 amino acid signal sequence plus a 79 amino acid mature region [7]. It circulates as a monomer, homodimer, and heterodimer with CXCL4/PF4. The monomer is considered the most bioactive, while the heterodimer is proposed to potentiate PF4 activity [8]. Mature human IL-8 shares 65% and 70% amino acid identiity with porcine and canine IL-8, respectively [9]. CXCR1 is (45-50) kDa in size and is used almost exclusively by IL-8. CXCR2 is (35-40) kDa in size and is used by almost all CXC chemokines [10]. Proteolytic processing is likely a cell-specific event and results in IL-8 N-terminal truncation. For example, fibroblasts and endothelial cells cleave amino acid 21 and 22 generating IL-8 [11]. CXCR2 responds to low concentrations of IL-8 and is principally associated with chemotaxis and MMP-9 release. CXCR1, by contrast, responds to high concentrations of IL-8 and is associated with respiratory burst and phospholipase D2 activation [12]. Interleukin 8 (IL-8) is a member of the CXC chemokine subfamily [13]. In vitro, IL-8 is produced by a variety of cells, including monocytes, alveolar macrophages, endothelial cells, and PMN [14]. A role for IL-8 in acute respiratory distress syndrome (ARDS) suggested further by recent data showing elevated levels in the airspaces of Corresponding author. Patients with ARDS or idiopathic pulmonary fibrosis or at risk of ARDS [15].

Materials and methods

Specimens collection

From May to December 2013, Fifty five specimen (blood) were collected in sterilized containers from pneumonia patients in three hospitals including : Educational Baghdad Hospital ,Educational Al-yarmook Hospital and private Nursing Hospital, and thirty specimens of blood from healthy persons was used as control, 2 ml of blood from each patient and control were put in sterilized container for WBC counts and 2ml of blood was put in plan tube then were centrifuged to obtaines serum.

Buffers

All reagents were bought to room temperature before used

Note: High concentrations of IL-8 found in saliva. It is recommended that a facemask and gloves be used to protect kit reagents from contamination.

1. Wash Buffer - If crystals have formed in the concentrate, warmed to room temperature and mixed gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

2. Substrate Solution - Color Reagents A and B mixed together in equal volumes within 15 minutes of usage. Protected from light. 200 mL of the resultant mixture is required per well. 3.Calibrator Diluent RD5P (1X) - Add 20 mL of Calibrator Diluent RD5P Concentrate (5X) to 80 mL of deionized or distilled water to yield 100 mL of Calibrator Diluent RD5P (1X).

4. IL-8 Standard - Reconstitute the IL-8 Standard with 5 mL of Calibrator Diluent RD5P (1X) (*for cell culture supernate samples*) or Calibrator Diluent RD6Z (*for serum/plasma samples*). This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 mL of Calibrator Diluent RD5P (1X) (*for cell culture supernate samples*) or Calibrator Diluent RD6Z (*for serum/plasma samples*) into each tube. the stock solution used to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (2000 pg/mL). The appropriate Calibrator Diluent serves as the zero standard (0 pg/mL).

Interleukin-8 (IL-8) assay

1. All reagents and working standards were prepared as directed in the previous sections.

2. The excess microplate strips removed from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

3. One hundred mL of Assay Diluent RD1-85was added to each well.

4. Fifty mL of Standard, control, or sample was added per well. Securely cover with a plate sealer and incubate for 2 hours at room temperature. Gently tap the plate to ensure thorough mixing.

5. Aspirated each well and washed, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 mL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last

wash, removed any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. One hundred mL of IL-8 Conjugate was added to all wells. Securely covered with a plate sealer and incubate for 1 hr. at room temperature.

7. Repeated the aspiration/wash as in step 5.

8. Two hundred mL of Substrate Solution was added to each well. Followed by incubation for 30 minutes at room temperature. Protected away from light

9. Fifty mL of Stop Solution was added to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if color change does not appear uniform, gently tap the plate should be done to ensure thorough mixing.

10. The optical density of each well was determined within 30 minutes, using a microplate reader set to (450 nm). If wavelength correction is available, set to (540 nm or 570 nm). If wavelength correction is not available, subtract readings at (540 nm or 570 nm) from the readings at (450 nm). This subtraction will correct for optical imperfections in the plate. Readings made directly at (450 nm) without correction may be higher and less accurate.

Results and discussion:

The serum level of IL-8 was assessed in pneumonia patients and controls group. As shown in table 1, the mean ± SE serum IL-8 concentration in the patients group was generally higher than control group,0.194 ± 0.046, 0.095 ± 0.006 pg/ml respectively, statistical significant at ($p < 0.05$).

Table 1-The mean concentration of IL-8 in pneumonia patients and control group

Groups	No.of cases	Mean ± SE
Patients	28	0.194 ±0.046
Control	27	0.095 ± 0.006
T-test value	----	0.061 *
* (P<0.05).		

In previous study, the results for IL-8 obtained strongly suggest that this cytokine, specifically produced in the lung, plays a significant role in the pathomechanism of a sustained fibrotic change of the lung, which we suspect to be a consequence of intraluminal organization [17].

In addition, there is a possibility that *M. pneumonia* has the ability to directly promote IL-8 production through the function of Toll-like receptors [18]. The results of current study showed IL-8 levels of patients group is higher than control group ,as shown in figure 1.
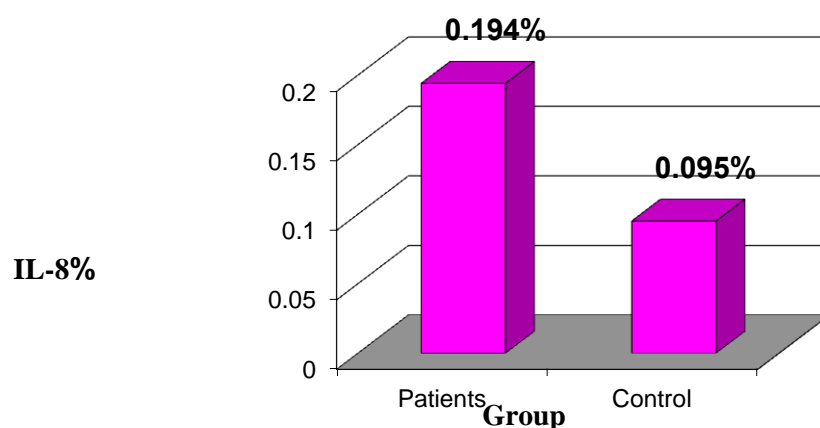

Figure 1-Comparism of IL-8 levels in pneumonia patients and control

Ventilator-associated pneumonia group had significantly higher concentrations of IL-8 in bronchoalveolar Lavage fluid than the control group. High BALF IL -8 concentration increased the post-test probability of VAP being present to 61.4% ,Multilevel likelihood ratios demonstrated that increasing levels of BALF IL-8 lead to increased confidence for "ruling in" VAP, with the highest

level examined (4000 ng/ml) producing a post-test probability of 75%[18]. Serum IL-8 levels were significantly elevated in patients with idiopathic interstitial pneumonia (IIP) ($P < 0.05$). In BAL fluid, the IL-8 level was significantly higher in patients with IIP ($P < 0.05$), hypersensitivity pneumonitis(HP) ($P < 0.01$). In several cases, IL-8 level correlated with neutrophil density, The IL-8 immunopositive cells were type II pulmonary epithelial cells and alveolar and interstitial macrophages in patients with IIP [19]. The number of patients with detectable serum levels of IL-8 was significantly higher among patients with acute chest syndrome (ACS) than in the control group (P   0.0001) the mean serum concentration of IL-8 was 6500 ± 3900 pg/ml. The mean serum IL-8 level was significantly higher in patients with ACS than the control group (P   0.04) [20]. The bronchoalveolar lavage (BAL) fluids from the three groups of patients contained significantly higher percentages of PMN relative to the healthy controls (76% ± 13%, 79% ± 11%, 74% ± 9%, and 2% ± 1%, respectively, in patients with the adult respiratory distress syndrome ARDS without pneumonia, ARDS with pneumonia, and pneumonia without ARDS and in controls, respectively ([mean ± SEM]; $P < 0.005$)[21]. The early appearance of IL-8 in BAL fluid of patients at risk of ARDS , high levels of IL-8 are present in the BAL fluid of ARDS patients with or without associated pneumonia and that levels are significantly higher than those of patients with pneumonia alone[22]. The presence of increased levels of IL-8 in the blood of patients with ARDS may play a role in the recruitment of neutrophils to the lung; indeed, it has been shown in vitro that IL-8 can increase the expression of L-selectin LAM-1, promoting integrin-mediated adhesion of neutrophils to endothelium [23].Moreover, although several authors have shown evidence of the role of IL-8 in neutrophil transendothelial migration in vivo [24]. a recent study showed that other factors such as endotoxins can inhibit neutrophil chemotaxis in response to IL-8 [25].

Also results was shown , the mean ± SE serum IL-8 concentration in childern age groups (2month- 3 years) were higher than age groups (15-60 years) and (60-85 years) , respectively, as Shown in table 2.

Table 2-Effect of age group in IL-8 levels

Age group	No. of cases %	Mean ± SE of IL-8
2 month-3 years	8	0.154 ± 0.04
15-60 years	13	0.131 ± 0.02
60-85 years	8	0.113 ± 0.2
LSD Value	---	0.114 NS
* (P<0.05).		

The percentage of age groups (2month-3years) were higher than age groups (15-60years) (60-85years), 0.154%, 0.131%, 0.133% respectively, as shown in figure 2.
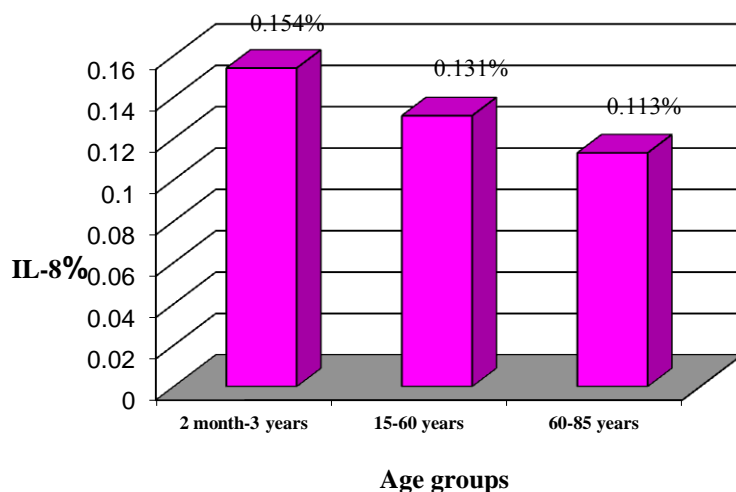

Figure 2-Correlation between pneumonia patients age and IL-8 levels

IL-8 was found to be elevated in 9 out of 10 samples of The bronchoalveolar lavage fluid(BALF) from children with acute chest syndrome (ACS). The mean level was 5500 ± 1400 pg/ml ,The child with the most severe presentation (patient 22) requiring intubation and mechanical ventilation had the highest BALF concentration of IL-8 at 180 000 pg/ml and is not included in the calculation of the mean. These levels were high compared with levels described in healthy adult controls, 21.5 pg/ml, and 59.5 pg/ml [26].

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