Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(7):403-408

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Serum interleukin-6 and C-reactive protein in bacterial otitis media patients in Damascus city

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ABSTRACT

Otitis media, an inflammation of the middle ear, is a common illness in childhood and also occurs in adults. This study aimed to investigate the correlation between chronic otitis media (COM) and acute otitis media (AOM) and the levels of serum Interleukin-6 (IL-6) and C- reactive protein (CRP) in patients contact clinics in Damascus hospital in period 2013–2014.This study involved 77 patients with otitis Media (OM), (COM: 45 vs AOM: 32) and 22 healthy people as a control group (age: 2-70 year). The results showed that both IL-6 and CRP were significantly increased ($p<0.05$) in serum of COM patients (Pseudomonas aeruginosa: 5.12±0.664 pg/ml, 6.72±0.624 mg/l) and in serum of AOM patients(Streptococcus pneumonia: 48.60±3.35pg/ml, 63.88±3.102mg/ l)as compared to healthy controls. The serum IL-6 and CRP levels from bacterial COM patients showed a little elevations. Contrarily, the serumIL-6 and CRP levels from bacterial AOM patients showed increased elevations especially in streptococcal AOM patients. Therefore, serum IL-6 and CRP would be a diagnostic biomarkers for bacterial otitis media.

Key words: Chronic Otitis Media (COM), Acute Otitis Media (AOM), Interleukin-6 (IL-6), C- reactive protein (CRP).

INTRODUCTION

Otitis media (OM) is the inflammation of the middle ear, which may be caused by bacteria, fungi or viruses. There are two main types of otitis media, acute purulent otitis media and chronic suppurative otitis media [1]. Otitis media is very common in childhood (2–5) years of age[2]. About75% of children experience at least one episode by their third birthday. Almost half of these children will have 3 or more ear infections during their first 3years. The reason for the higher frequency in these populations is the anatomic differences in skull base and Eustachian tube and biologic susceptibility[3]. Although OM is primarily a disease of infants and young children, it can also affect adults[4]. Furthermore, the incidence is higher in males than in females[5]. The significant risk factors in OM include socioeconomic status, cultural, seasonal, and age factors, as well as family history of middle ear disease. The most common bacterial pathogens of OM are Streptococcus pneumoniae, Hemophilus influenza and Moraxella catarrhalis. Other pathogens responsible for OM are Staphylococcus aureus, Escherichia coli, Klebsiella species, Pseudomonas aeruginosa and Proteus species. The types of pathogens involved in OM have also been found to be dependent on geographical location[1].

The Otitis media (OM) is an inflammatory response to either acute or persistence stimuli typified by accumulation of both cellular and chemical mediators in the middle ear[6]. These mediators, which are proteins, glycoproteins, peptides, cytokines, prostaglandins, leukotrienes and others, have a role in the inflammatory process of the middle ear such as vascular permeability changes, chemotaxis, stimulation epithelial activity secretory, enhancement of mucous glycoprotein secretion, and production of other mediator. OM is frequently caused by bacteria (However, cases of sterile OM are not uncommon) which enter the middle ear from the nasopharynx via Eustachian tube. In cases of bacterial OM, the source of bacteria is currently believed to be nasopharyngeal tonsils (adenoids).

Endotoxin, a compound of bacterial cell walls, is believed to be responsible for initiating inflammatory in the middle ear.In addition, keratinocytes have the ability to produce many soluble mediators independently from immune cells in response to injury, including, IL-1, IL- 6 and IL-8[6, 7]. Many of these cytokines contribute to the acute phase of the inflammatory response, prime the immune system for rapid activity, and promote the release of other cytokines.

Interleukin-6 is a proinflammatory cytokine that plays a key role in the acute phase reaction, including the induction of CRPC-reactive protein (CRP) is a predominantly synthesized by the hepatocytes, under transcriptional control by the cytokine interleukin 6. Several authors have reported that IL-6 is a sensitive and reliable marker of neonatal bacterial infection. Interestingly, in a study of children with AOM, serum sample from the AOM patients infected with Streptococcus pneumonia contained significantly higher levels of IL-6 than those infected with other bacteria or no bacteria [8]. It appears that Streptococcus pneumonia stimulates IL-6 production more than other bacteria.Previous study have demonstrated that serum levels of c-reactive protein are higher in bacterial than in no bacterial AOM , but the usefulness of CRP as a screening test is limited because its sensitivity and negative predictive value are inadequate for clinical purposes [8, 9].

EXPERIMENTAL SECTION

Sampling: Hospital and private clinics in Damascus City were the area selected for the study, because of large number of attending there. Pus or purulent discharges from cases of otitis media were collected from patients having the disease using sterile swab stick. The diagnostic of AOM and COM based on a questionnaire which was filled by the physicians for each patient in their clinics. At the same time, blood samples were obtained from these patients for determination of IL-6 and hs-CRP. The blood samples were centrifuged immediately, the resultant serum was distributed in aliquots which were stored at -80C until assays were done.

Identification of bacterial strains

Identification and characterization of the bacterial strains were carried out as described by Cowon and Steel (2002) after they have been examined using such biochemical tests as Gram stain, Catalase test, Indole test, Oxidase test, Coagulase test, Methyl red Test, Urease test and Optochin Sensitivity test, And subculturing on selective or/and deferential media such as blood agar, macConke agar, EMB agar, chapman agar and TSI agar.

Assays: Serum levels of IL-6 were determined by using Enzyme-Linked Immunosorbent Assay (ELISAkits). The IL-6 Human ELISA Kit (R&D System, USA) utilizes an antibody specific for human IL-6 coated on a 96-wellplate. The standard curves were based on dilution of recombinant human IL-6, the concentration of work solutions were 100- 50- 25- 12.5- 6.25- 3.12 pg/ml. Absorbance is measured at 450 nm. Serum hs-CRP concentrations were determined by nephelometry (Human kit, Germany).The assay is based on a latex-enhanced turbidimetric immunoassay method. This agglutination is detected as an absorbance change (570 nm), with the magnitude of the change being proportional to the quantity of CRP in the sample.

Statistical analysis: data were analyzed using Excel (2010) and SPSS version 20. Results were presented as mean ±SD. Comparisons between the means of two independent groups were performed using Student's t-test. Person coefficient for studying correlations between parameters, and $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

A total number of otitis media patients were 87, patients who have positive bacterial cultures were 77 (88.50%),selected to be the population in our study, and 10 patients (11.5%) have negative bacterial cultures, not selected. The bacterial etiology of AOM and COM were determined by routine bacterial cultures. The pathogenic bacterial strains in COM patients were Pseudomonas aeruginosa (44.45%), Staphylococcus aureus (31.12%), Staphylococcus epidermidis (4.44%), Proteus (13.33%) and Klebsiella pneumonia(6.66%).And the pathogenic bacterial strains in AOM patients were Streptococcus pneumonia (65.63%), Hemophilus influenzae (9.83%), Staphylococcus aureus(9.83%) and Pseudomonas aeruginosa (15.63%).Tables (1) and (2) demonstrate the pathogenic bacterial strains and their proportionality within AOM and COM patients.

Table 1. Distribution of COM pathogenic bacterial strains

%	N	COM pathogenicbacterial strains
44.45%	20	*Pseudomonas aeruginosa*
31.12%	14	*Staphylococcusaureus*
4.44%	2	*Staphylococcus epidermidis*
13.33%	6	*Proteus*
6.66%	3	*Klebsiella pneumoniae*
100.00%	**45**	**Total**

Table 2. Distribution of AOM pathogenic bacterial strains

%	N	AOM pathogenic bacterial strains
65.63%	21	*Strep. pneumoniae*
9.38%	3	*Hemophilus influenzae*
9.38%	3	*Staph. aureus*
15.63%	5	*Pseudomonas aeruginosa*
100.00%	**32**	**Total**

Interleukin-6 (IL-6) and C-reactive protein were measured in stored serum samples from 77 patients with otitis Media (OM) (Chronic OM: 45 verses Acute OM: 32) and from 22 healthy persons as a control group. Out of 45 with Chronic OM cases, 31 patients (60.78%) were adults above 40 years old (age: 41-70 year). And out of 32 with Acute OM, 23 patients (63.89%) were children (age: 2-5 years). Tables 3 and 4 indicate the distribution according to ages for Chronic OM and Acute OM consecutively.

Table 3. Distribution of COM patients according to ages

%	N	COM patients according to ages
7.84%	4	6 - 20years
15.69%	8	13 -20 years
5.88%	3	21-30 years
9.80%	5	31- 40 years
60.78%	31	41- 70 years
100%	**51**	total

Table 4. Distribution of AOM patients according to ages

%	N	AOM patients according to ages
63.89%	23	2 - 5 years
30.56%	11	6 - 12 years
5.56%	2	13- 20 years
100%	**36**	total

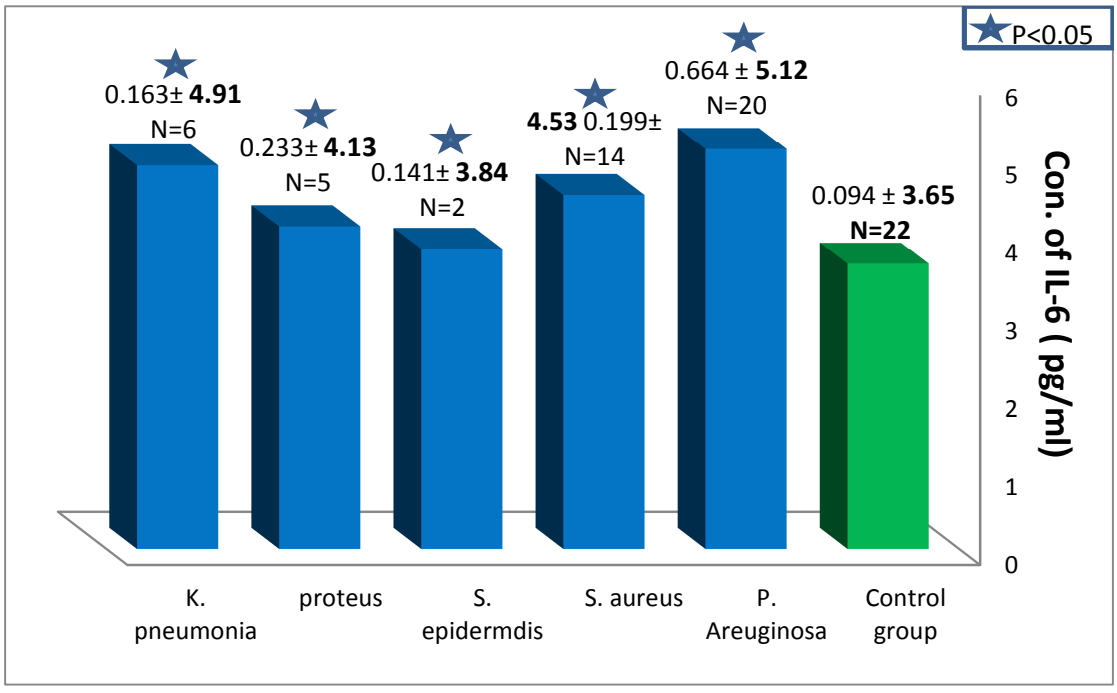

Figure (1) related outcomes of statistical results for IL-6 in chronic bacterial otitis media patients vs control group

The **IL-6** concentrations means (pg./ml) for each bacterial strains group were compared with that of control group by T-test and P-value. In COM group, the mean concentration of IL-6 was, for: Pseudomonas aeruginosa infected patients 5.12 pg./ml±SD 0.664,Staphylococcus aureus infected patients 4.53 pg/ml±SD 0.199,Staphylococcus epidermidisinfected patients 3.84 pg/ml ± SD 0.141,Proteusinfected patients 4.13 pg/ml ± SD 0.233,Klebsiella pneumoniae infected patients 4.91 pg/ml ±SD 0.163. P-Value were below 0.05 in all cases. In AOM group, the

mean concentration of IL-6 was, for: Strep. pneumoniae infected patients 8.17 pg/ml ±SD 3.35, Hemophilus influenzae infected patients 48.60 pg/ml ±SD 1.095, Staph. aureus infected patients 4.17 pg/ml ±SD 0.351, Pseudomonas aeruginosa infected patients 6.34 pg/ml ±SD 0.304.P-Value were below 0.05 in all cases except in Staph. aureus infected patients group was above 0.05.In control group, the mean concentration of IL-6 was: 3.65 pg/ml ±SD 0.094,figures(1) and (2).

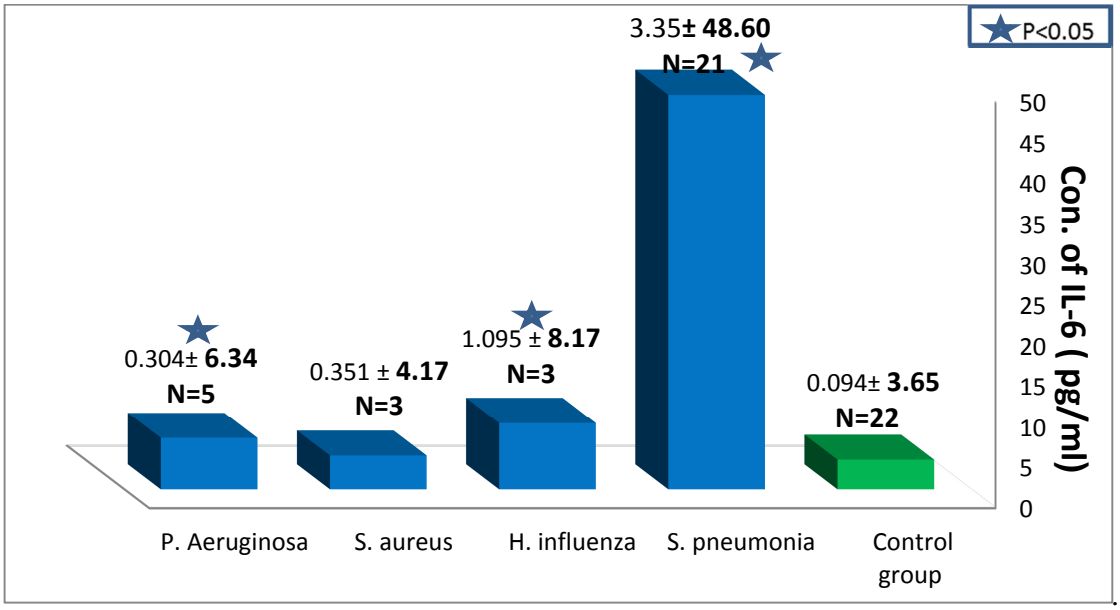

Figure (2) related outcomes of statistical results forIL-6 in acute bacterial otitis media patients vs control group

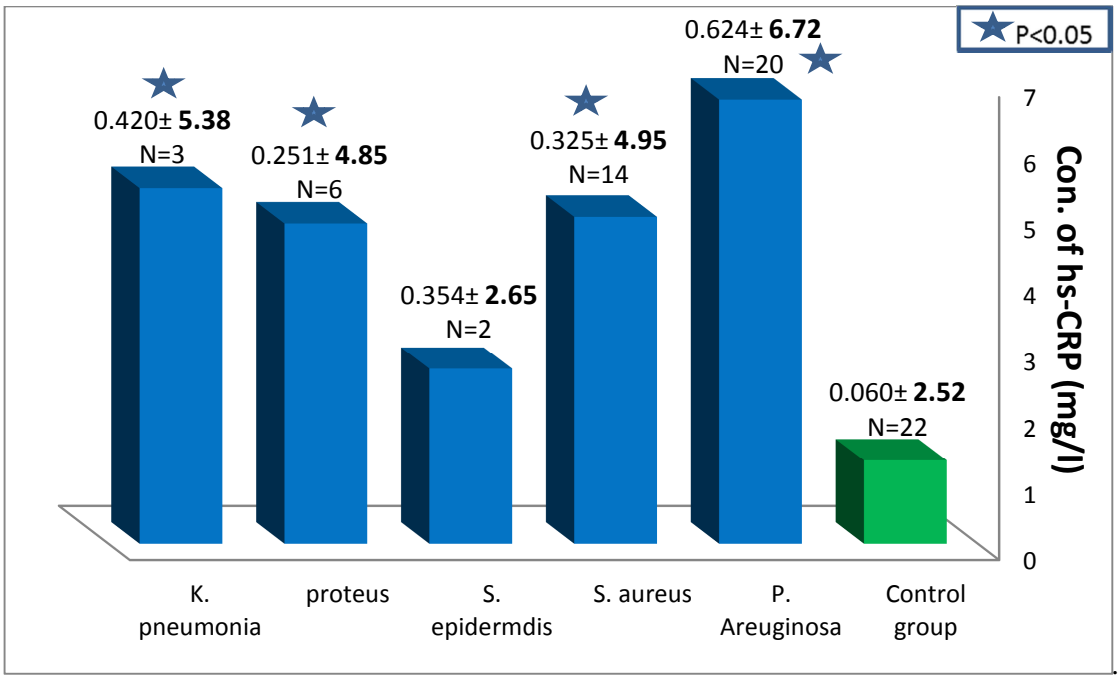

Figure (3) related outcomes of statistical results for CRP in chronic bacterial otitis media patients vs control group

The **hs-CRP** concentrations means (mg/l) for each bacterial strains group were compared with that of control group by T-test and P-value. In COM group, the mean concentration of hs-CRP was, for: Pseudomonas aeruginosa infected patients 6.72 mg/l ±SD 0.624, Staphylococcus aureus infected patients 4.95 mg/l ±SD 0.325, Staphylococcus epidermidis infected patients 2.65 mg/l ±SD 0.354, Proteus infected patients 4.85 mg/l ±SD 0.251, Klebsiella pneumoniae infected patients 5.38 mg/l ± SD 0.420. P-Value were below 0.05 in all cases except in Staphylococcus epidermidis infected patients group was above. In AOM group, the mean concentration of hs-CRP was, for: Strep. Pneumoniae infected patients 63.88 mg/l ±SD 3.102, Hemophilus influenzae infected patients 10.73

mg/l ±SD 0.185, Staph. aureus infected patients 5.48 mg/l ±SD 0.161, Pseudomonas aeruginosa infected patients 8.33 mg/l ±SD 0.174. P-Value were below 0.05 in all cases. In control group, the concentration of hs-CRP was: 2.52 mg/l ±SD 0.060,figures (3) and (4).

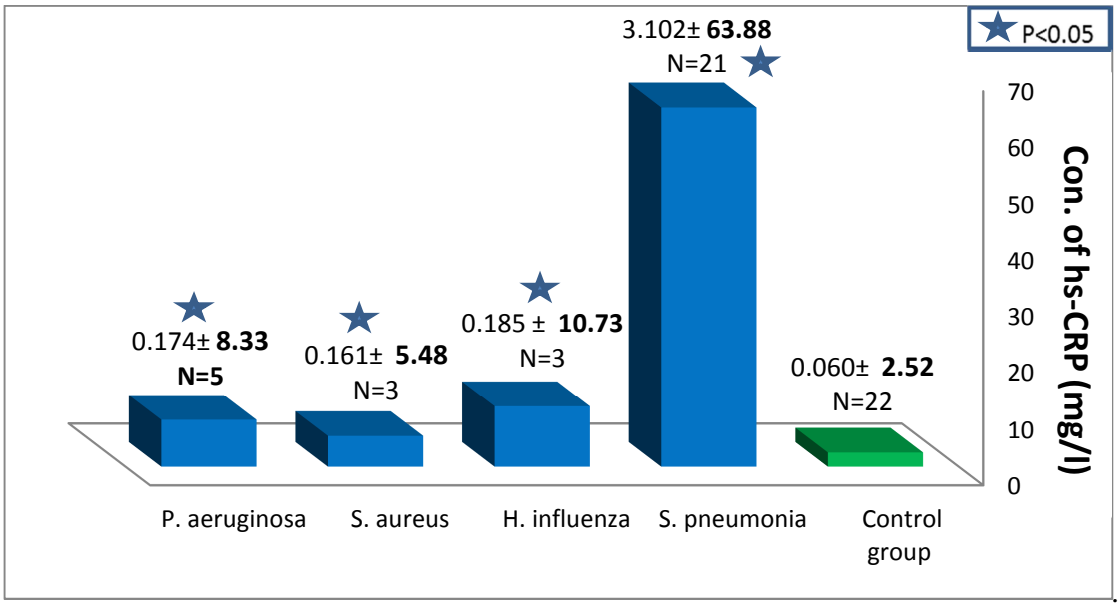

Figure (4) related outcomes of statistical results forCRP in acute bacterial otitis media patients vs control group

IL-6 in COM and AOM

In comparison study (figure 1) concerning IL-6 concentrations in COM patients vs controls, there were little elevations (1.5 times of control group 3.65pg/ml), but statistically significant (P<0.05). These low levels of IL-6 don't rule out bacterial etiology of COM, especially the main bacterial pathogens Pseudomonas aeruginosa and Staphylococcus aurous which were confirmed in certain studies[5, 10]. Therefore, IL-6 is not enough as a biomarker of bacterial COM .

Contrarily, serum IL-6 levels (figure 2) in AOM patients caused by streptococcus pneumoniae were significantly higher (mean, 48.60 pg/ml vs. 3.65pg/ml, P<0.05) than IL-6 levels in AOM caused by Haemophilus influenza (8.17 pg/ml) or Pseudomonas aeruginosa (6.34pg/ml),P<0.05. Previous study of Mellus A and all demonstrated the expression of cytokine gen during pneumococcal and haemophilus influenza AOM in the rate[11].Our findings were in agreement with those obtained by Heikkinen T. and all. whether for causative pathogens, specifically streptococcus pneumonia, or for the serum value of IL-6 in children (40.1 pg/ml)[8]. Therefore, the IL- 6 serum value would be a predictive marker for severity or chronicity of OM. Whereas, The little elevation (1.7 to2.2 times of the control group but statistically significant P<0.05), in IL-6 levels in AOM patients caused by Haemophilus influenza (8.17 pg/ml) or Pseudomonas aeruginosa (6.34pg/ml) would not be a predictive marker for severity or chronicity of OM, that means these two bacterial strains did not produce enough IL-6. The ability of pneumococcal AOM to induce a significant high serum IL-6 than that of Haemophilus influenza or Pseudomonas aeruginosa is interesting. A more likely explanation for the differences is that the inflammatory process in the middle ear during pneumococcal AMO may be more severe than that caused by the two other principal AMO pathogens. Serum IL-6 levels >30 pg/ml could be highly specific for pneumococcal AOM, in agreement with study [8].

Hs-CRP in COM and AOM

In comparison study (figure 2) concerning hs-CRP concentrations in COM patients vs controls, there were little elevations (1.9to 2.6 times of control group), but statistically significant (P<0.05, except patients with S.epidermidis, P>0.05). The correlation between these elevations and the bacterial pathogenic agents were positive within patients with Pseudomonas aeruginosa (median 6.72 mg/l vs 2.52mg/l of control group; P<0.05), Klebsiella pneumonia (5.38 mg/l (P<0.05), Staphylococcus aureus (4.95 m/l P<0.05), but was negative within patients with Staphylococcus epidermidis (2.65 mg/l P>0.05).The bacterial etiology of COM was in agreement with previous studies[1, 12].

In AOM patients, there were a significant elevations in hs-CRP levels correlated with the bacterial strain pathogenic agents; streptococcus pneumonia were significantly higher (63.88 mg/l vs 2.52 mg/l of control group; P<0.05) than that of Haemophilus influenza (10.73 mg/l; P<0.05) or Pseudomonas aeruginosa (8.33 mg/l;P<0.05) or Staphylococcus aureus (5.84 mg/l; P<0.05). Our results were in agreement with that of previous study (Murphy T. et

al, 2006) especially the bacterial pathogens streptococcus pneumonia and haemophilus influenza[8, 13].According to the data from the National Health and Nutrition Examination survey (NHANES), the median serum CRP is 2.2 mg /l in adults and 0.4 mg/l in children under age 20.Johanis and all, found in children with bacterial infection the CRP value was 14.06 mg/l, our results is in agreement with this study [14].

CONCLUSION

We demonstrated increased serum levels of IL-6 and CRP in bacterial OM patients with an excellent diagnostic value given by IL-6 concentrations and a good one given by CRP in the differentiation between healthy people and OM patients. Both IL-6 and CRP can be used as biomarkers for diagnostic purpose and high levels of IL-6 and CRP could differentiate streptococcal OM from other bacterial OM.

Acknowledgement
The authors are sincerely thankful to Dr. Michael Abed, Biochemistry and Microbiology Department, SPU University, Syria, for his useful advices and help. The authors also thank Mr. Ossama Mohssen for the statistical analysis.

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