European Review for Medical and Pharmacological Sciences 2012; 16: 1033-1038

The relationships between clinical outcome and the levels of total antioxidant capacity (TAC) and coenzyme Q (CoQ10) in children with pandemic influenza (H1N1) and seasonal flu

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Abstract. – BACKGROUND AND AIM, This study was planned to evaluate the relationships between the levels of total antioxidant capacity (TAC) and Coenzyme Q (CoQ10) and clinical outcome in hospitalized children with pandemic influenza (H1N1).

Serum copper (Cu) and zinc (Zn) levels were also determined to evaluate the changings of oxidative stress's enzyme activities depending on their cofactor concentrations.

PATIENTS AND METHODS, Children with suspected H1N1 virus infection were hospitalized and nasal swabs were sent to laboratory for confirmation of H1N1 by rRT-PCR assay. Age and sex matched 31 healthy children were included as Control Group. Total antioxidant capacity and CoQ10 were determined by spectrophotometry and HPLC, respectively, and Cu and Zn were determined using atomic absorption spectrometer.

RESULTS, Totally 28 children had H1N1 and 37 children had seasonal influenza (SI). TAC, CoQ10 and Zn levels were found to be significantly decreased in H1N1 patients (1.01 ± 0.19, 752.2 ± 163, 69 ± 27, respectively) compared to Control Group (1.64 ± 0.36, 934 ± 21, 92 ± 4, respectively). Seasonal Influenza group had significantly decreased TAC and Zn levels (1.31 ± 0.27, 78 ± 34 respectively) compared with control group (1.64 ± 0.36, 92 ± 41, respectively). CoQ10 levels were also found as decreased in H1N1 compared to seasonal influenza (752.2 ± 163 vs 1022 ± 199, $p = 0.003$). There was a significant correlation between CoQ10 levels of sera and chest radiographic findings of patients with H1N1 pneumonia. No significant differences were found in serum Cu levels between patients with H1N1 and SI or control group (150 ± 45 vs 127 ± 37, $p = 0.215$).

CONCLUSIONS, Pandemic influenza infection had increased oxidative stress compared to the seasonal influenza.

Key Words:
Flu, Total antioxidant capacity, Coenzyme Q, Copper, Zinc.

Introduction

A novel virus was detected in several patients showing influenza-like illness at April 2009, in California[1]. Virus was found to be an H1N1 virus that was genetically and antigenically unrelated to human seasonal influenza viruses but genetically related to viruses circulating in swine[2] and this has had a major impact on antigenic evolution[3]. The emergence of mutations in the viral genome is of ongoing public and scientific concern, because such changes may affect the infectivity, pathogenicity and antigenicity[4]. Some mechanisms involved in the clearance of pathogens, such as the generation of reactive oxygen species (ROS) by phagocytes, which could participate in the development of the disease[5]. Oxidative stress is defined as a disturbance of the pro-oxidant/antioxidant balance in favor of pro-oxidants; it is more likely that increased production of oxidants may have caused the decrease in the antioxidants and oxidative damage to macromolecules may contribute to the pathogenesis of the disease. Coenzyme Q10 (Co Q10) is a potent antioxidant and an obligatory coenzyme for mitochondrial enzyme complexes in oxidative phosphorylation for the production of adenosine triphosphate (ATP). CoQ10, synthesized endogenously in humans, is known for its key role in mitochondrial bioenergetics, inhibits protein and DNA oxidation and the peroxidation of cell membrane lipids and also that of lipoprotein lipids present in the circulation. Because of its importance in the development of tolerance to oxidative stress, CoQ10 has been the focus of many researches because of varying degrees of CoQ10 deficiency cause oxidative stress[6]. CoQ10 is now known to play a significant protective role in several pathological conditions[7-9].

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Zinc (Zn) and copper (Cu) are essential micronutrients for enzymes that catalyze oxidation–reduction reactions[10]. Oxidative processes occur most intensively in the background of an imbalance of trace elements incorporated into the structure of enzymes responsible for antioxidant protection[11]. Both increase and decrease of trace element ion contents can affect the activity of the antioxidant enzymes[12]. It is reasonable to assume that trace elements will be exerting actions, directly or indirectly, on the influenza pathogenesis that is not stated previously.

The levels of antioxidants can already be measured separately in the laboratory, but these measurements are time-consuming, labor-intensive and costly. The number of different antioxidants in all biological samples makes it difficult to measure each antioxidant separately. Measurement of total antioxidant capacity (TAC) is a useful test for prediction of oxidative total status[13]. Total antioxidant status (TAS) may be an important factor providing protection from oxidative damage caused by H1N1 related oxidative stress. According to best of our knowledge, no studies have been published previously about the oxidative stres parameters in H1N1.

Therefore, we hypothesized that the development of influenza viral pneumonia may lead to the depletion of antioxidants and protective enzymes such as CoQ10, thus contributing to disease severity[14]. To assess the potential importance of oxidative stress and damage in influenza; we determined the levels of TAC, CoQ10 status and Cu and Zn as trace elements that related to antioxidant enzymes.

Patients and Methods

The study has been carried out in accordance with the Declaration of Helsinki (2008) and approved by the Ethics Committee of Dicle University Medical Faculty.

Subjects

All children admitted to the Dicle University Hospital (Diyarbakir, Turkey) from November 2009 to January 2010 with influenza like signs and symptoms [ILSS] and suspected of H1N1 infection were enrolled in this study. ILSS were defined as a fever and upper respiratory tract symptoms (cough, sore throat, rhinorrhea, congestion), lower respiratory symptoms (wheezing, chest pain, shortness of breath), or gastrointestinal symptoms (abdominal pain, vomiting, diarrhea). Twenty-eight children had H1N1 and 37 children had seasonal influenza (SI). Clinical and/or radiological pneumonia were detected in 24 of H1N1 and four patients died. Thirty-one healthy children were included to the study as control group.

Samples

H1N1 virus infection was confirmed by National Influenza Reference Laboratory with the detection of the viral genomes with the real time reverse transcription polymerase chain reaction (RT-PCR) using a nasal swab specimen obtained at the time of admission. All respiratory virus samples were collected via nasopharyngeal aspirate (NPA) by trained nursing staff. Negative H1N1 results regarded as seasonal influenza. Control group was selected from healthy children who were examined for routine check-up with permission of the parents. None of the patients had been vaccinated. Exclusion criteria for SI were positive bacteriological throat or urine culture and rheumatologic diseases. Sera were collected at the time of hospital admission with high fever.

Biochemical Analysis

Each collected blood sample was immediately centrifuged at 4000 rpm +40°C for 10 min and then transferred into an Eppendorf tube. Samples were transferred on ice and kept in –70°C deep-freeze until the end of the study which was completed within three months. Total antioxidant capacity were measured by Erel's methods with Abbott Architect c16000 (Abbott Park, North Chicago, IL, USA) chemistry analyzer[13]. Zn and Cu were measured by Shimadzu 6401S atomic absorption/emission spectrometer (Shimadzu Biotech, Kyoto, Japan). The acetylene flow rate and the burner height were adjusted in order to obtain the maximum absorbance signal with a slit of 0.5 nm, at a wavelength 213.9 nm for Zn and 324.8 nm for Cu. The radiation sources were hollow cathode lamps (Shimadzu Biotech, Kyoto, Japan). The operating conditions were those recommended by the manufacturer[15]. CoQ10 levels were determined by reversed-phase high-performance liquid chromatography with ultraviolet detection (RP-UV-HPLC) (Agilent 1100, Agilent Technologies, GmbH & Co. KG, Waldbronn, Germany)[16]. The HPLC system was Agilent 1100 series and reagent kit was Chromsystems (Chromsystems, Instruments and Chemicals, Munich, Germany) including internal standard. CoQ10 was dissociated from lipoproteins by

Table I. Descriptive and clinical features of study groups.

	Controls (n = 31)	SI (n = 37)	H1N1 (n = 28)
Median and ranges of age (month)	52 (3-152)	48 (2-160)	59 (4-168)
Male (n)	17	16	15
Female (n)	14	22	13
Cough (n)	1	21*	26*
Diarrhea (n)	1	1	7*,†
Vomiting (n)	0	3	9*
Dyspnea (n)	0	15*	16*

SI: Seasonal influenza
*$p < 0.05$ compared with control, † $p < 0.05$ between SI and H1N1.

methanol and subsequently cleaned-up on silica gel and octadecyl silica solid-phase extraction cartridges. The mobile phase consist of 0.01 M ammonium acetate (pH4.0)-methanol (35:65, v/v) with an Chromsystems (1509/5) column (5 microm, 4.6 mm × 150 mm) at a flow rate of 2,5 ml/min, column temperature 25°C and detection wavelength 275 nm.

Statistical Analysis

The SPSS statistical package vs 11.5 was used to perform all the statistical analyses (SSPS Inc. Chicago, IL, USA). Distribution pattern of data was assessed by Kolmogorov-Smirnov test. Student's *t* test was used to evaluate the differences between independent two groups. Kruskal-Wallis test and Spearman's correlation analysis was used to investigate the relationship and correlations recpectively between Q10 levels and pneumonia severity. For all analyses, a *p* value of less than 0.05 (two-tailed) was considered significant.

Results

Of the 28 H1N1 patients, 8 were followed up in the Intensive Care Unit (ICU), and four of them died. Descriptive and clinical features of the patients are shown in Table I. Age of the patients ranged from 48 days-old to 14 years-old. The mean age of children was 4.5 ± 2.9 years. Pneumonia was detected in 24 of H1N1 based on the clinical and/or radiological findings. Serum oxidative stress parameters of both groups are documented in Table II. There were no significant differences in age and gender distribution between study groups ($p > 0.05$). Total antioxidant capacity, CoQ10 and Zn levels were found to be decreased significantly ($p = 0.003$, $p = 0.011$, $p = 0.018$ respectively) in H1N1 patients compared to seasonal influenza patients (Table II). Significantly decreased TAC and Zn levels were found in children with H1N1 compared with the controls ($p = 0.009$, $p = 0.024$ respectively) (Table II).

Table II. Comparison of serum oxidative stress variables in studied groups (mean±standard deviation).

	Controls (n=31)	SI (n=37)	H1N1 (n=28)	Difference P1	P2	P3
TAC (μmol H_2O_2 Equiv./L)	1,64 ± 0.36	1.31 ± 0.27	1.01 ± 0.19	0.009	0.003	0.006
Q10 (μmol/L)	934 ± 217	1022 ± 199	752.2 ± 163	0.141	0.011	0.003
Cu (μg/dL)	136 ± 52	127 ± 37	150 ± 45	0.342	0.175	0.215
Zn (μg/dL)	92 ± 41	78 ± 34	69 ± 27	0.024	0.018	0.142

TAC: Total antioxidant capacity, H1N1: H1N1 Influenza A virus infection, SI: Seasonal influenza, CoQ10: Coenzyme Q, P1: Difference between SI and Controls, P2: Difference between H1N1 and Controls, P3: Difference between H1N1 and SI.

Differences of Cu levels (Table II), white blood cells count (WBC) and the other routine laboratory tests were not found as statistically significant between two groups ($p > 0.05$).

Increasing severity of pulmonary involvement was found as together with significant decrease of serum CoQ10 levels in H1N1 but not in SI (Table III). There was no relationship between mortality and biochemical parameters.

Discussion

The main symptoms in the influenza virus diseases arise from the cytopathic effects in the epithelial cells lining of the respiratory tract, and additionally from the release of immune active mediators, which trigger the cascade of events directed towards elimination of the virus[17]. Reactive oxygen species are believed to be responsible for many of the pathological changes that occur in pneumonia[18]. ROS could contribute to the tissue damage seen in lungs either directly by oxidizing lipids, proteins and nucleic acids[19,20]. In present study, decreased TAC and Zn levels in patients with influenza showed that the increased production of ROS associated with influenza may exceed the capacity of the total antioxidant defense system, resulting in oxidative damage to selected proteins, lipids, and DNA.

There were no significant change in Cu levels in patients with influenza or clinic status. This shows that antioxidant enzymes related with Zn take more intense role other than enzymes related with Cu. Contrary to rapid course of influenza infection late response of Cu related enzymes to the infection may be related to this situation.

It is stated that subtypes of influenza viruses induce different pro-inflammatory gene expression which determine the severity of diseases such as H5N1 versus H1N1. This proinflammatory stages lead to different pro oxidative stress responses[21,22]. So, regulation of some enzymes that take part in oxidative defense system may be different. In our investigation CoQ10 was decreased significantly in patients with H1N1 but not in SI. This situation may be related with pathogenesis of H1N1. CoQ10 is important particularly for the peroxidation of cell membrane lipids and also that of lipoprotein lipids present in the circulation. Lipid oxidation may be of importance in the pathogenesis of H1N1 infection because of a decrease in the concentration of antioxidants which may result in local immunosuppression[23]. This decrease was observed in our study (Table II).

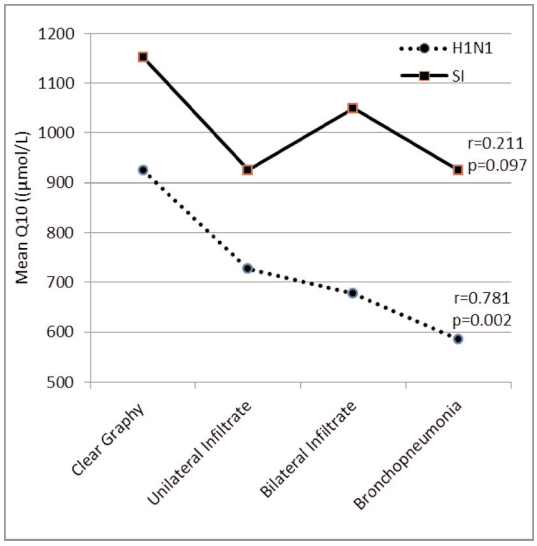

Figure 1. Median Q10 levels were correlated with pneumonia severity (r = 0.781, $p = 0.002$) in H1N1 but not in SI (r =0.211, $p = 0.097$). Clear graphy means graphic not showed any infiltrate.

Table III. Serum CoQ10 levels (µmol/L, mean ± SD) and number of patients with different roentogenographic findings.

X-ray findings	Controls		SI		H1N1	
	n	mean ± SD	n	mean ± SD	n	mean ± SD
Clear graphic	31	941 ± 205	5	1101 ± 288	4	911 ± 223
Unilateral infiltrate	-		14	935 ± 213	13	721 ± 197
Bilateral infiltrate	-		12	1013 ± 261	7	674 ± 159
Bronchopneumonia	-		6	942 ± 217	4	589 ± 171

CoQ10 levels were decreased in patients with unilateral infiltrate, bilateral infiltrate and bronchopneumonia (*$p = 0.012$, 0.009, 0.001 respectively) compared to patients with clear graphic. Q10 levels were also decreased in patients with bronchopneumonia compared patients with unilateral infiltrate, bilateral infiltrate (†$p = 0.021$, 0.014 respectively). SD: Standard deviation.

A duality was found between inflammation and ROS contributing to the disease severity, reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), the hydroxyl radical (OH) and the superoxide anion (O_2-), are produced during tissue injury which are known to contribute to the severity of several diseases[24]. At the same time lipid oxidation may be the key pathway in pathogenesis of H1N1 because the chest radiographic severity was highly correlated with depletion of CoQ10 in H1N1 but it was not the case in SI (Figure 1, Table III). Our findings showed a clear association between host antioxidant capacity and the probability of H1N1 infection in agreement with the literature[25].

The antioxidant effects of zinc are generally manifested in the presence of a demonstrable short-term increase in levels of this metal sulfhydryl stabilization and reduction in the formation of ·OH from H_2O_2 and O_2 – through the antagonism of redox-active transition metals[26]. In our study, decreased Zn levels were associated with H1N1 and it has been previously shown that Zn deficiency is relevant to H1N1 influenza[27]. Zn is also involved in coordination of zinc finger peptides which are important in inhibition of viral transcription[28]. Therefore, prophylactic use of Zn may be effective against H1N1 virus infection and should be further explored as an option for treating human influenza virus infections[29].

In conclusion, pandemic influenza H1N1 infection has an increased oxidative stress compared to the seasonal influenza.

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