

## **Liver function tests abnormality in influenza H1N1 in Southeastern of Iran.**

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### **Abstract**

**Novel influenza A/H1N1 virus has a pandemic outbreak all around the world and makes a serious concern for global health organ systems. While, elevation in liver function tests is observed in associate with contaminating with influenza A/H1N1. The study was aimed to evaluate the liver function tests abnormality in Influenza H1N1 in south-eastern of Iran. 330 patients who referred to Ali-ibn Abi Talib Hospital, Zahedan University of Medical Science, Iran with the primary symptoms of Influenza A/H1N1 were participated. All hospitalized patients were subjected for nasopharyngeal RT-PCR for pandemic H1N1 virus. Also, liver function tests including AST (Aspartate Aminotransferase), ALP (Alkaline Phosphatase), ALT (Alanine Transaminase), PT (Prothrombin Time), PTT (Partial Thromboplastin Time), TBIL (Total Bilirubin) and DBIL (Direct Bilirubin) were done for patients. At the end statistical analysis was performed in order to compare the results from both RT-PCR test and liver tests. Spss version 20 was used to analyse the data and data were evaluated by chi-square test. 150 persons were positive for H1N1 by RT-PCR. The values of AST, ALP, PT and PTT were increased statistically but TBIL and DBIL showed no significant difference. In conclusion, we forcefully supposed a close relationship between liver function and immune system elements.**

**Keywords:** Influenza A/H1N1, Respiratory tract infections, Liver function tests, Liver injury.

*Accepted on July 12, 2016*

### **Introduction**

The spread of the influenza A/H1N1 virus in 2009 has indubitably been a great challenge for the global health systems because the seasonal influenza virus is famous for its fast mutation rate and outbreak. To perceive these alterations, a large amount of studies has been done to discover the genomic variation [1,2]. Moreover, many new epitope regions of basic HA antigen will have evaluated by seasonal changes. As a result, various types and subtypes of influenza virus generally shown in different human society [3,4].

The first cases of human infection epidemic A /H1N1 virus were recognized in the United States in April 2009 [5,6]. At the same time, a prevalence of this virus was identified in Mexico [7] and then the virus found in other regions. Based on the clinical survey of hospitalized patients for influenza A/ H1N1, this variant of virus has a preference to sickening younger population [8,9]. By June 2009, the World Health Organization (WHO) publicly declared the first pandemic of the 21st century 2 months later.

The influenza A/H1N1 virus usually influences the respiratory tract endothelium, and after appearance of its symptoms the shedding takes 2-5 days [10]. Real-Time Polymerase Chain Reaction (RT-PCR) was performed on the nasopharyngeal

secretion for respiratory infection. On the other hand, a large amount of clinical studies has been published to clarify the clinical manifestations during the influenza A/H1N1 infection, and preparing sufficient data which indicate the multi-organ involvement [11]. As a result, not only influenza A/H1N1 has the specific impairment of the respiratory function, also it reveals a negative effect on the function of other vital organs, such as the liver, kidneys, gastrointestinal tract and central nervous system [12-15]. In addition, there are some researches on mouse models propose more different organs localization than hypothesized previously, such as the lung, heart, liver and spleen [16]. Regarding laboratory papers, the values of the Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Total Bilirubin (TBIL), Direct Bilirubin (DBIL), and haemostatic tests including Prothrombin Time (PT) and Partial Thromboplastin Time (PTT) assume as the significant screening examinations for an abnormal liver function [17].

Here in, we performed a comprehensive clinical study based on relationship between respiratory diseases of influenza A/H1N1 associated with alteration liver function by performing RT-PCR to detect positive H1N1 virus infection and enzymatic liver test for assessment of liver dysfunction.

## Materials and Methods

This study was conducted on 330 patients in the range of 1 month to 18 years old consist of 72 male and 78 female; among them 45 persons had less than 1 year, 72 of them had the age ranged between 1 and 10, and 33 persons were between 10 to 18 years old. The study was performed according to the Helsinki principals and is approved by ethical committee of the Zahedan University of Medical Sciences and all authors signed a written constant. The diagnosis was made in Ali-ibn Abi Talib Hospital, Zahedan University of Medical Sciences, Iran. All people showed primary symptoms of Influenza A/H1N1 such as cough, sore throat, myalgia or influenza-like illness, fever, nausea, and headache. All hospitalized patients were subjected for nasopharyngeal RT-PCR for pandemic H1N1 virus with the use of Swine H1N1 influenza Human pandemic strain kit according to the manufacturer's instructions; first, through specific primers and probes for the detection of influenza type A, a viral infection was diagnosed and then by using specific primers and probes for subtypes of swine influenza, H1N1 diagnosis was performed. Genes related to protein detection of subtype H1N1 were Hemagglutinin (HA) and Neuraminidase (NA). Also, liver function tests including AST, ALP, ALT, PT, PTT, TBIL and DBIL were done for patients. The PT test is performed by adding the patient's plasma to some source of Tissue Factor (e.g. a protein, thromboplastin, from homogenized brain tissue) that converts prothrombin to thrombin. The mixture is then kept in a warm water bath at 37°C for one to two minutes. Calcium chloride (excess quantities of ionized calcium) is added to the mixture in order to counteract the sodium citrate and allow clotting to start. The test is timed from the addition of the calcium chloride until the plasma clots. The aPTT test uses blood which is decalcified to prevent clotting before the test begins. The plasma is separated by centrifugation. (Ionized) Calcium and activating substances are added to the plasma to start the intrinsic pathway of the coagulation cascade. The substances are: kaolin (hydrated aluminium silicate) and cephalin. Kaolin serves to activate the contact-dependent Factor XII, and cephalin substitutes for platelet phospholipids. The partial thromboplastin time is the time it takes for a clot to form, measured in seconds [18]. At the end Statistical analysis was performed in order to compare the results from both RT-PCR test and liver tests. Spss version 20 was used to analyse the data and data were evaluated by chi-square test.

## Results

We observed 330 persons who admitted to Ali Ibn Abi Talib hospital for epidemic A/H1N1. Real-Time Polymerase Chain Reaction (RT-PCR) was used to confirm the diagnosis. Through all patients, 150 persons were positive for H1N1 by RT-PCR, while 180 persons were negative. Besides, all patients were both positive and negative on the testing of RT-PCR were subjected for liver function tests to confirm a relationship between respiratory complications infection and the liver injury. All proportions were calculated as percentages of the patients with available data are shown in Table 1. An increased

value of AST, ALT, PT and PTT test were observed and statistically seemed meaningful. While, no significant differences were observed in clinical outcomes for TBIL and DBIL tests.

**Table 1.** Association between H1N1 influenza status with liver enzyme status\*.

Enzyme	Status	H1N1		Total	C.C	P value
		Positive	Negative			
AST	+	51 (34)	27 (15)	78 (24)	0.217	0.000
	-	99 (66)	153 (85)	252 (76)		
	Total	150 (100)	180 (100)	330 (100)		
ALT	+	48 (32)	18 (10)	66 (20)	0.264	0.000
	-	102 (68)	162 (90)	264 (80)		
	Total	150 (100)	180 (100)	330 (100)		
ALP	+	39 (26)	36 (20)	75 (23)	0.071	0.195
	-	111 (74)	144 (80)	255 (77)		
	Total	150 (100)	180 (100)	330 (100)		
TBIL	+	18 (12)	14 (7.78)	32 (10)	0.071	0.197
	-	132 (88)	166 (92.22)	298 (90)		
	Total	150 (100)	180 (100)	330 (100)		
DBIL	+	9 (6)	7 (3.89)	16 (5)	0.049	0.374
	-	141 (94)	173 (96.11)	314 (95)		
	Total	150 (100)	180 (100)	330 (100)		
PT	+	42 (28)	18 (10)	60 (18)	0.226	0.001
	-	108 (72)	162 (90)	270 (82)		
	Total	150 (100)	180 (100)	330 (100)		
PTT	+	24 (16)	9 (5)	33 (10)	0.18	0.001
	-	126 (84)	171 (95)	297 (90)		
	Total	150 (100)	180 (100)	330 (100)		

\* Data are presented as N (%).

The PT (Prothrombin Time) and PTT (Partial Thromboplastin Time) measured finishing time from coagulation cascade activation and used to assess unexplained clotting. In some children the PT and PTT prolonged without any clinical symptoms of bleeding, which could indicate a liver dysfunction [19]. Also in influenza A/H1N1 the average level of Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) increased [20] in terms of abnormal

hepatic functions. Alkaline Phosphatase (ALP) is a protein found in all body tissues. The liver is one of tissues with high amounts of ALP. In the case of liver disease or hepatitis an increased level of ALP would be observed [21].

## Discussion

Influenza A/H1N1 virus has been considered as a main reason for the respiratory infection; however its pathogenesis is not completely recognized. Based on some publications, there would be a remarkable reason to believe that influenza A/H1N1 virus can influence other organs because of an immune reaction to viral antigens [22]. So, we performed a clinical study including young generation from geographically diverse setting across Zahedan region to assess the above mention relationship. According to obtained result, there was a specific elevation in liver function test (AST, ALT, ALP, PT and PTT) in association with respiratory infection. This data could be support by the result of Chen Yingying [23] mentioned abnormal liver tests and reduced ability of the liver in protein synthesis in people who were tested positive for epidemic A/H1N1 virus. Also, Zarogoulidis et al., [24] reported abnormal values of the liver enzymes in H1N1-positive patients and liver malfunction. PApic et al., [22] suggested a dependency between liver enzyme elevations with the hypoxemia. Based on the Fislova' studding H1N1 virus disseminated from the lung to other organs through a process of transient viremia to indicate a high chance of possibility for multi-organ involvement due to respiratory H1N1 infection. What is more, Influenza A/H1N1 could trigger a stormy release of cytokines which could directly harm liver during influenza A/H1N1 infection and should be regarded as a potentially hepatotropic [25,26].

In conclusion, we forcefully supposed a close relationship between liver function and immune system elements. However, more studies are needed, to assess the pathophysiology of hepatitis due to H1N1 infection, and to explain a logical pathway based on monitoring of multi-organ involvement due to the H1N1 virus.

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