Original Article

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Molecular Detection of H1N1 and Impact of Cytokines among Infected Patients with Respiratory Distress: A Cross-sectional Study

Microbiology Section

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ABSTRACT

Introduction: Viruses are spread from one individual to another. In respect to the mode of transmission, the majority of them enter the human body by the inward breath of infective respiratory beads. Upper Respiratory Tract Infections (URTI) are infections of the body's respiratory tract, which include the sinuses, nose, throat, airways, and lungs. The influenza virus has four key structural antigens: the internal Ribonucleoprotein (RNP), the viral envelope Matrix (M), and two surface Glycoproteins (GP), Neuraminidase (NA) and Haemagglutinin (HA). A respiratory virus called swine flu/H1N1 evolved and spread widely around the world. H1N1 outbreaks with various virus strains were noted before the most recent severe pandemic, which occurred in 2009. Seasonal outbreaks that are extremely infrequent since the 2009 pandemic the influenza strain have occurred.

Aim: To assess the prevalence and level of cytokines in H1N1-infected patients with signs and symptoms of respiratory distress.

Materials and Methods: This is a cross-sectional study of viral molecular, immunological and epidemiological parameters. The study was done at the Indian Council of Medical Research (ICMR) at the Department of Health Research (DHR), Virus Research and Diagnostic Laboratory (VRDL), Department of Microbiology, Burdwan Medical College and Hospital (BMCH), Burdwan, India. The duration of the study was seven months, from January 2022- July 2022. Samples in Viral Transport Medium (VTM) were collected from suspected influenza patients with mild or severe Acute Respiratory Distress Symptoms (ARDS) and Influenza Like Symptom (ILS). The RNA samples were isolated and the nucleic acid purified from samples was screened by Real-time Polymerase Chain Reaction (RT-PCR). The extracted clinical Ribonucleic Acid (RNA) samples are then converted into Complementary Deoxyribonucleic acid (cDNA). The HA gene sequences of endemic swine influenza A virus (H1N1) and sequences from a panel of human and avian type A influenza virus strains, including the type A human seasonal strains, were retrieved from the GenBank database in the National Centre for Biotechnology Information (NCBI) portal. The Enzyme-linked Immunoassay (ELISA) method was used to measure the proinflammatory cytokines Interleukin (IL)-8, IL2 and anti-inflammatory cytokine IL10, as well as the IL3 concentration level in severe Acute Respiratory Distress Syndrome (ARDS) +Immediate Life Support (ILS) positive H1N1 infected patients. Analysis of all the data was performed by Statistical Package for Social Sciences (SPSS) software version 22.

Results: In this study, all samples (n=120) were examined using RT-PCR, which revealed that 53 samples were infected with the Influenza A Virus (IAV). Among the total positive 24 were males and 29 were females. the average concentration of IL3 was 1749.49 pg/mL. The increase in IL8 was not as big as the increase in IL2 and IL3. IL8 was identified as a significant proinflammatory factor during angiogenesis, or uncontrolled cell growth.

Conclusion: The prevalence of H1N1 infection was found to be high in children under the age of ten. The concentration of IL-3 in H1N1-infected patients' samples was higher than the concentrations of the other three cytokines.

Keywords: Influenza, Interleukins, Osteoclastogenesis, Real-time polymerase chain reaction, Swine flu

INTRODUCTION

Most viruses that pose serious public health risks are spread from person to person. In terms of transmission, most of them enter the human body by the inhalation of infective respiratory droplets. As a result, infections spread easily within intimate populations. Shifts in recent global trends such as extended travel, urbanisation, exotic pet breeding, deforestation, and religious traditions all contribute to the easy spread of modern viruses from one region of the world to the next [1].

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Influenza A (H1N1), Respiratory Syncytial Virus (RSV), Human Metapneumovirus, Parainfluenza virus 1, 2, 3, 4, Adenovirus, and Rhinovirus are the viruses that cause Respiratory Tract Infections (RTI) the most often. After the COVID-19 pandemic, the Novel Coronavirus emerges as one of the most important causes of RTI. Based on differences in the antigenic structure of Ribonucleoprotein (RNP) and Matrix (M) proteins, influenza viruses are divided into three types: A, B, and C [2].

Isolation of viral particles before dispersion remains a difficulty since, they travel through numerous routes in a very short period. The point here is that an outbreak stays undiscovered until clinical signs occur, because of these reasons or on these reasons the virus spans national and continental boundaries. When an outbreak occurs, a high alarm is sent through numerous channels of communication throughout the world. During each epidemic, the World Health Organisation (WHO) and the Centres for Disease Control and Prevention (CDC) implement a variety of risk reduction and prevention methods. The WHO proposes effective vaccination plans for public use every year, the most recent being in February 2019. The most recent significant pandemic caused by H1N1 occurred in 2009, before which outbreaks with different strains of the virus were observed. Since the 2009 pandemic influenza strain has generated rare seasonal outbreaks [3].

Influenza A (Family Orthomyxoviridae, Genus Influenza Virus A is the most serious pandemic illness threat to humanity today. Its competitors for this title (HIV-1, Ebola, SARS, pneumonic plague) have greater untreated fatality rates but lack influenza's quick inter-personal transmission and seasonal dispersion. A new pandemic epidemic would result in around 135 million fatalities globally during the first year. Outside of mammals, disease containment is thus

nearly impossible. It may also be transmitted between mammals, and the current influenza A H1N1 2009 pandemic is thought to have started in pigs [4,5].

A total of 12,604 people were tested till August 20th, out of which 2401 tested positive for influenza and H1N1 (swine). A substantial number of cases are now being reported from Maharashtra (Mumbai and Pune), Karnataka (Bangalore), and Tamil Nadu (Chennai), India. Most of these occurrences affected young individuals who were otherwise healthy. 36 instances with test confirmation of death have allegedly occurred. The bulk of people who passed away had some underlying medical conditions and had arrived late at the designated medical institution. A novel subtype of the influenza A (H1N1) virus identified by genetic sequencing had elements from the human, avian, swine, and swine viruses from North America and Eurasia [6].

The virus can stimulate the activation of immune cells (such as T cells, B cells, macrophages, dendritic cells, neutrophils, and monocytes) and resident tissue cells, leading to the generation of high quantities of inflammatory cytokines. When the flu virus is present, cascade amplification events of interferon-stimulated gene expression trigger the innate immune response. The interferon (IFN) is largely produced by monocytes, macrophages, and dendritic cells [7]. It has been suggested that enhanced responses to infection, such as fever, phagocytic cell recruitment, and blood vessel permeability, are associated with up-regulated levels of IL1, IL6, IL12, and IL23. Based on the following well-known data, it was thought that, increased cytokine production following P(H1N1) infection was a significant factor in the pathophysiology and progression of the disease. Invasion of the P(H1N1) influenza virus causes the release of proinflammatory cytokines, which leads to the onset of pneumonia in animals. IFN-, IL4, IL5, and IL10 production increased in mice infected with P(H1N1) virus. IL6 and IL18 levels were greater in P(H1N1)-infected macaques compared to non infected ones. Early on in the pandemic H1N1 influenza virus infection, the innate immune system becomes active with increased production of the cytokines IL2, IL12, IFN-, IL6, TNF-, IL5, IL10, IL17, and IL23. Serum IL6 and IL10 levels increased with illness severity in individuals with severe pandemic H1N1 influenza. In the severe P(H1N1) patient. The pathological bone degradation that comes along with osteolytic inflammatory disorders and physiological bone remodelling is brought on by osteoclasts. Numerous clinical disorders, even those that only affect the bone locally, are characterised by inflammation. Under pathological circumstances, a number of cytokines are produced. These cytokines (IL1, IL6, IL7, IL8, IL11, IL15, IL17, IL23, IL34 and Transforming Growth Factor-β (TGF-β) cause osteoclast development and bone resorption directly, as well as osteocyte death because of the production of osteoclastogenic cytokines and osteocyte expression of osteoclastogenic cytokines. Osteocyte apoptosis, which produces osteoclastogenic cytokines, is enhanced during bone resorption [8].

Hence, this study gives a definitive direction to the clinicians and researchers about the role of IL effects in H1N1 infection in ARDS and ILS patients. The study was conducted for the first time at Burdwan Medical College, Burdwan, India, on the immunological role in influenza viral infection among those patients who has admitted with severe and mild ARDS, and ILS.

MATERIALS AND METHODS

This institutional based cross-sectional study was conducted at the Department of Microbiology, Burdwan Medical College, West Bengal, India during the tenure of January to July 2022. A total of 120 throat swabs and nasal/nasopharyngeal swabs were collected in a Viral Transport Medium (VTM). The study has been approved by the Institutional Ethics Committee (IEC) of Burdwan Medical College. The memo number is BMC/IEC/303.

Sample size calculation: The sample size in this study was purposive so, there is no defined sample size. The patients were selected as per the Ministry of Health and Family Welfare guidelines (MOHFW) for categorising H1N1 cases. Category B and C patients were subjected to testing for H1N1 [8]. Samples were submitted with a properly completed proforma that included information on the patient's demographics, symptom onset date, co-morbidities etc.

Inclusion criteria:

- An acute respiratory illness with measured fever of ≥38 °C and cough, with onset within the last 10 days.
- Only individuals who represented symptomatic and asymptomatic acute respiratory infection with fever, cough, sore throat, runny and stuffed nose, bodyaches, headache, chills and fatigue.
- Only individuals who showed manifestations of dehydration and Gastrointestinal (GI) disorders.
- Patients with both mild and severe ARDS and ILS.

Exclusion criteria:

- Pregnant women.
- SARS-CoV-2 positive patients.
- Individuals who had severe allergic reactions.
- Patients who were not willing to give samples were also excluded from the study.

Study Procedure

The Oropharyngeal (OP) and Nasopharyngeal (NP) swabs was collected in, VTM with the help of flocked non toxic synthetic fibres such as polyester as well as synthetic nylon handled swabs sticks.

Viral RNA isolation: The patient's RNA from NP-OP swabs contained in VTM was isolated by using the HiPurA Viral Automated RNA purification Kit (HiMedia). RNA concentration was measured by measuring OD values using a spectrophotometer.

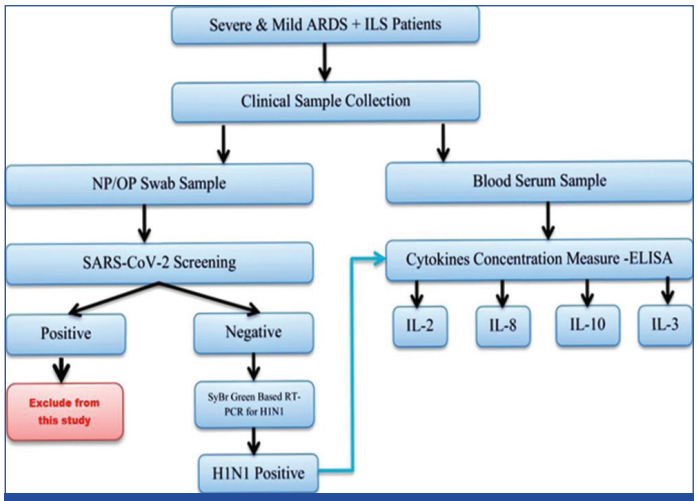
RT-PCR for SARS-Cov2 screening: The nucleic acid purified from samples was screened by real-time PCR. In this process, COVIPATH™ Applied Bio-System Kit was used as per manufacture's guidelines, which runs the test by using two probes for two different target sequences which were specific to SARS-CoV-2 Open Reading Frame of 1ab (ORF1ab) gene and nucleocapsid protein (N) gene, and one target sequence that was specific to RNase P. After interpretation of the result, positive samples are excluded in this molecular detection. SARS CoV-2 negative were further processed along with the history of ARDS and ILS.

C-DNA synthesis: The extracted clinical RNA samples then convert into cDNA by Prime Script™ 1st strand cDNA Synthesis Kit 6110A (Takara Bio) as per the manufacturer's protocol.

Oligonucleotide design and synthesis: The HA gene sequences of endemic swine IAV H1N1, and sequences from a panel of human and avian type A influenza virus strains, including the type A human seasonal strains, were retrieved from the Gen Bank database in NCBI portal. The primers were designed from NCBI Primer-Blast online platform portal in respect of A/Homosapiens/India/171/2020(H1) strain. The forward primer sequence was 5'-AAGAAGTCCTCGTGCTATGG-3' and the reverse primer was 5'-CGTGGACTGGTGTATCTGAA-3'. The amplicon length of the HA-designed primer was 309bp. Another primer set was used here for the Neuraminidase (NA) gene in respect of A/India/H1N1p2/2020(H1N1) strain of influenza virus. Forward and Reverse primers are 5'-CGTCCCAAAGATGGAACAGG-3' and 5'-TCTAGCCCTGTTAGCTCAGG-3', respectively.

Molecular Detection of HINI by real-time PCR (RT-PCR): The SYBR green qualitative Real Time-PCR (RT-PCR) reactions that target region of HA and NA gene of H1N1 were performed using TB Green Premix Ex Taq II (Ti RNase H Plus) manufactured by TAKARA Bio. Each reaction consisted of 1X TB Green Premix master mix,

designed primers of a short sequence of H1N1 HA gene in a 25 µL reaction. Bio-Rad CFX 96 real-time PCR conditions consisted of initial denaturation incubation at 95°C for 2 minutes followed by 38 cycles of alternating 95°C incubations for 5 seconds, 56°C incubations for 30 seconds, and 72°C incubations for 10 seconds. Fluorescence was detected after every 72°C extension incubation. A total of 120 clinical samples were screened by this method where the Influenza A virus (H1N1) can be confirmed. To assess each specimen's DNA suitability for PCR amplification, the presence of the housekeeping gene Beta Actin was employed as an internal control. Here in this study Beta Actin, used to validate the SYBR Green based RT-PCR test run, which act as an internal test run control [Table/Fig-1].



[Table/Fig-1]: Flowchart of the exclusion and inclusion criteria of this study along with workflow.

Immunological study-Pro and anti-inflammatory cytokines: Serum interferons, cytokines, and chemokines in patients with H1N1-induced pneumonia and ARDS are abnormally increased, indicating a cytokine storm [7]. The severity of the disease affects the degree and type of immunological dysregulation found in these people; nonetheless, higher levels of IFN-gamma, IL6, IL1, IL1, TNF-, IL15, IL12p70, IL17, IL10, MCP-1, MIP-1, IL8, MIG, IP-10, MIP-1, GM-CSF, and RANTES are the most often reported results [9,10]. It seems comparable when the neutrophil-to-lymphocyte is huge the ratio [10,11]. Unsurprisingly, the onset of severe multiorgan tissue malfunction and destruction is associated with the H1N1 cytokine storm [12].

Patients' blood serums were collected and stored at -80°C. Cytokines were quantified by specific Human ELISA kit sets (Krishgen Biosystems) following the manufacturer's instructions. In this study, authors have measured the concentration of only four cytokines that potentially impact the human immune system. The Interleukins were measured and grouped into two categories according to their functions: ProInflammatory cytokines IL8 (KB1068) and IL2 (KB1064), and anti-Inflammatory cytokine IL10 (KB1072). IL3, which promotes acute inflammation, was also discovered [13]. All test samples including standards were measured as per manufacture's guideline, Krishgen Biosystems and measured the OD values with the help of ELISA Reader (Manufactured by: J MITRA).

The study was done during the third wave of the pandemic in India. The duration of this study was seven months, from January to July 2022. So, the clinical signs and symptoms were similar to SARS CoV-2 infection. A mixed infection of SARS-CoV2, the common cold, and ILS was observed significantly during the time frame of the current study, which was conducted from January 2022 to July 2022, when the atmospheric temperature occurred in a wide range. This study was carried out as a comparison between COVID-19 and IAV infection, as the signs and symptoms of COVID-19 and IAV infection are very similar. Hence, during the time of the molecular study, they were distinguished into 2 parts: one is SARS-CoV2, and the other is IAV. After exposure of viral infection the cytokine storm generated and elevated all the cytokines and chemokines level of concentration in the human body [14].

The H1N1 infection can develop pseudo-COVID symptoms, which mask up some COVID-19 negative samples. Patients without SARS-CoV-2 infections have some respiratory distress along with fever, cough, sneezing, and bodyaches. So, the first phase involved identifying H1N1-infected patients from COVID-19 negative samples, followed by an immunological study using four (4) types of cytokines, namely proinflammatory (IL2 and IL81), anti-inflammatory (IL10) and acute inflammation responsible interleukin (IL3). The selection of interleukins in the 3 categories was done randomly in this study. Patients with acute or mild ARDS with ILS were selected from SARI and FLU Clinic wards from Burdwan Medical College and Hospital.

STATISTICAL ANALYSIS

Analysis of all the data was performed by SPSS software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Qualitative variables were expressed as mean±Standard Deviation (SD).

RESULTS

Molecular detection and patients' characteristics: This pandemic occurred in 2009 and spread to Iran as well as the whole world. From January 2022 to July 2022, a total of 120 samples were tested. All samples (n=120) were collected from admitted patients at Burdwan Medical College and Hospital. RT-PCR analysis revealed that 53 of 120 (44.17%) samples were IAV positive [Table/Fig-1,2]. Among the total positive, 24 males (45.28%) and 29 females (54.72%) patients' outcome were voluntarily illustrated in [Table/Fig-3]. The positive patients were divided into seven age groups. The first group lies between 0 and 10 years of age, and 20 individuals (37.73%) were found to be present in this group. For that, Group 2, Group 3, Group 4, Group 5, Group 6, and lastly Group 7 were 7.54%, 11.32%, 16.99%, 5.66%, 11.32%, and 9.43% IAV positive, respectively.

Parameters	Positive (n=53)	Percentage (%)
Sex		
Male	24	45.28
Female	29	54.71
Age (years)		
≤10	20	37.73585
11-20	4	7.54717
21-30	6	11.32075
31-40	9	16.98113
41-50	3	5.660377
51-60	6	11.32075
>60	5	9.433962
Sign and symptoms		
Cough	42	79.24528
Fever	46	86.79245
Runny nose	32	60.37736
Sore throat	24	45.28302
Wheezing	33	62.26415
Ear pain	17	32.07547
Bodyaches	30	56.60377
Headache	18	33.96226
Diarrhoea	27	50.9434
Vomiting	22	41.50943

Co-morbidities		
Bronchial asthma	17	32.07547
Endocrine and metabolic diseases	15	28.30189
Gastrointestinal (GI) disorders	17	32.07547
Atopic dermatitis	12	22.64151
Diabetes	15	28.30189

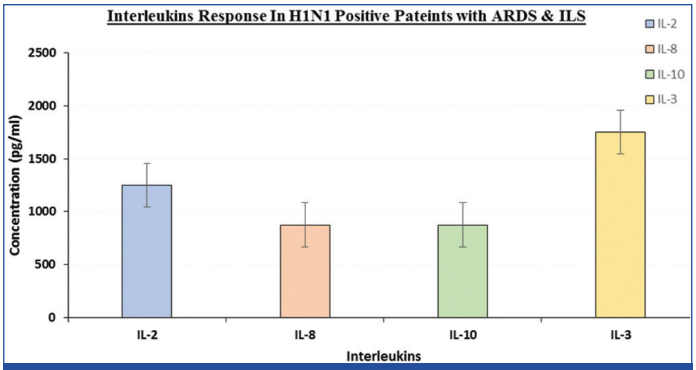
[Table/Fig-2]: Prevalence of Influenza A virus (H1N1) infection and outcome in H1N1 positive patients.

The prevalence of H1N1 infection in patients with underlying diseases was higher compared with those without underlying diseases. Underlying diseases and co-morbidities such as bronchial asthma, endocrine, and metabolic diseases, GI disorders, atopic dermatitis, and diabetes, were shown higher number in H1N1-infected patients compared with non infected ones. The most prevalent signs and symptoms were: cough in 42 patients (79.24%), fever in 46 patients (86.79%), wheezing in 33 patients (62.26%). This study demonstrated that the risk of contracting the H1N1 virus was significantly influenced by underlying illnesses or co-morbidities different parameters with their prevalence percentage were listed in [Table/Fig-2].

Interleukins response in H1N1 detected patients: In this study, 24 severe ARDS+ILS patient blood samples from 53 H1N1 IAV positive by RT-PCR cases were evaluated the concentration level of interleukins in serum sample. The rest of them were combinations of asymptomatic, with mild ARDS and no sign of ILS. The average concentration of IL3 which induces acute inflammation was 1749.49 pg/mL of blood. Simultaneously, IL2 and IL8 which categorises into proinflammatory cytokines found at 1249.322 pg/mL and 874.595 pg/mL average concentration in the blood, respectively. In a group of Anti-Inflammatory, Cytokines IL10 shows 874.595 pg/mL which is the same as IL8. The illustration was represented in [Table/Fig-3] with the SD result. Total P1–P24 all patients' cytokine concentrations in their blood serum are evaluated by the ELISA method and results are listed in [Table/Fig-4].

Patient S. No.	IL-2 Conc. (pg/mL)	IL-8 Conc. (pg/mL)	IL-10Conc (pg/mL)	IL-3 Conc (pg/mL)
P1	1297.286	1234.28	618.2857	618.2857
P2	1403	1345.68	922.5714	922.5714
P3	1311.571	1305.68	645.4286	645.4286
P4	1491.571	1309.68	398.2857	398.2857
P5	1417.286	1418.48	664	664
P6	1407.286	1463.28	831.1429	831.1429
P7	1377.286	1415.68	1246.857	1246.857
P8	1693	1384.08	966.8571	966.8571
P9	1381.571	1291.68	435.4286	435.4286
P10	1354.429	1393.28	731.1429	731.1429
P11	1347.286	1360.88	542.5714	542.5714
P12	1454.429	1324.48	1028.286	1028.286
P13	1545.857	1400.48	1106.857	1106.857
P14	1291.571	1512.88	1172.571	1172.571
P15	1290.143	1426.88	1326.857	1326.857
P16	1527.286	264.48	1069.714	1069.714
P17	2364.429	28.48	1421.143	1421.143
P18	3723	1378.88	1252.571	1252.571
P19	2001.571	1336.88	826.8571	826.8571
P20	1747.286	868.48	969.7143	969.7143
P21	1545.857	1251.68	591.1429	591.1429
P22	2195.857	1486.88	846.8571	846.8571
P23	3143	1423.28	756.8571	756.8571
P24	2675.857	1357.28	618.2857	618.2857

[Table/Fig-3]: Cytokines concentration in H1N1 positive patients with ARDS and ILS.



[Table/Fig-4]: Interleukins response in H1N1 positive patients with ARDS and ILS of 24 individuals (n=24).

DISCUSSION

During the 2009 influenza pandemic, just a few instances of human H1N1 infection were documented throughout West and Central Africa, including Nigeria. The virus has been circulating globally as a seasonal human influenza virus in the postpandemic period [15]. Indeed, due to the pandemic virus's dominance over other seasonal influenza viruses in many countries, the pandemic virus has been routinely recommended as a component of both trivalent and tetravalent influenza vaccinations in recent years [16]. A/Michigan/45/2015 (H1N1) pdm09-like virus has been suggested as a component of both trivalent and tetravalent vaccinations for the 2017-2018 northern and southern hemisphere influenza seasons [17]. The pandemic 2009 A (H1N1) virus is a novel virus containing gene segments from various swine and avian influenza viruses. As a result, the human population appears to have little protection against the virus [18]. The pandemic of IAV, took place in 2009, has generated a mild antiviral response which is reflected by a weak induction of IFN genes. Tumour Necrosis Factor (TNF) -expression was also low in primary human Human Dendritic Cells (DCs). A powerful cytokine response is typically linked with highly pathogenic influenza viruses such as the pandemic 1918 A (H1N1) and avian H5N1 viruses [19].

In this study, all the 120 clinical samples that were negative for SARS-CoV-2, were further analysed by RT-PCR through SYBR Green for IAV. Among which 53 (44.17%) samples were positive for IAV. Similarly, a study conducted by Pandita AK et al., has found the positivity rate for H1N1 virus is 30%. A similar study conducted at Rajasthan reported 39.3% positivity rate [20]. The majority of the positive patients in this study were from the age group of ≤10 years (37.74%), followed by the age group of 31-40 years (16.98%). Unlikely, another study conducted in Uttarakhand, India, revealed that the highest number of H1N1 infection rate is among the age group of 41-50 years (21.9%). They have reported only 9.1% H1N1 positivity rate below 10 years of age [20]. In this current study, the H1N1 positive female patients outnumbered the male one. Unlikely some other studies reported male preponderance [20,21]. The most common clinical presentations in this study were fever, cough, sore throat and bodyaches. Similarly, these findings have been coincided with some other studies where they have been reported the common signs and symptoms are fever, cough, sore throat, and bodyaches [20,22]. It is very evident that various co-morbidities can significantly worsen the disease trajectory and the present study also affirms the same. In this current study, it is very evident that the comorbidities like GI disorders (32.07%), bronchial asthma (32.07%), diabetes mellitus (28.3%) have worsen the disease condition and progression [Table/Fig-1]. Similarly, another study have strengthen these findings regarding the correlation between the worsening disease condition and comorbidities, where they have reported hypertension (29%) and Diabetes mellitus (18.3%) and COPD/asthma (10.9%) were the leading comorbidities [20]. Patients with ARDS+ILS were the main focus of this study because their mortality rate was high in previous research articles. In this study, it was founded that the IL3 and IL2

concentration level was elevated as a comparison to the other two cytokines IL8 and IL10. A study conducted by Yu et al., where IL2 concentration was lower than IL8 and IL10 concentration (pg/mL) [23]. The elevated IL3 levels in MM patients' bone marrow plasma were substantially higher than in normal controls. IL3 may cause Osteoclast cells (OCL) development in human bone marrow cells at concentration levels comparable to those detected in Multiple myeloma (MM) patient samples, and OCL formation generated by MM patient marrow plasma can be reduced by employing an anti-IL3 blocking antibody [24]. IL3 also indirectly affects osteoclastogenesis by boosting the effects of Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) and Macrophage inflammatory protein 1-alpha (MIP-1a) on OCL growth and development. It directly increases MM cell proliferation [25] and inhibits Osteoblast (OBL) production via a factor generated by macrophages in the bone marrow microenvironment [26].

IL3 mainly proinflammatory cytokines connects the immune system's T-lymphocytes, which detect foreign material invasion, and the haemopoietic system, which develops the cellular components that mediate defences and repair responses. IL3 is also generated fast after mast cell or eosinophil activation, such as through cross-linking Fc receptors, and may stimulate the development of T helper cell type 2 (TH2) responses via its actions on a subset of dendritic cells [27]. IL3 stimulates the widest number of haemopoietic system targets of any cytokine, and it also has the unique capacity to stimulate the formation of early stem cells, mast cell progenitors, and megakaryocytes [28]. In the future, IL3 antagonists may offer novel therapies for allergy and inflammatory illnesses. This study provides hospital-based epidemiological data, but wider community-based studies are warranted to attain a more precise and accurate understanding of influenza A H1N1. Besides this IL2 and IL8 are also reported to be proinflammatory cytokine which is also elevated in the patient's body [29]. Though the elevation of IL8 is not significant enough as IL2 and IL3. As IL8 was reported to be a major proinflammatory agent during angiogenesis or uncontrolled cell growth [29]. Therefore, the lower elevation of such in comparison with the others indicated that the infection might not promote uncontrolled cell growth or so. On the other hand, IL10 is considered a potent anti-inflammatory cytokine that strongly inhibits the production of proinflammatory cytokines [30,31]. The results from this study, suggested a down regulation of IL10 signifies the promotion of acute inflammation in the patient's body.

Limitation(s)

The lack of controls and for all the patient data for some variables were not obtainable. In this current study, the other respiratory viruses that cause URTI or Lower Respiratory Tract Infection (LRTI), apart from H1N1, were not included. The data for this current study were obtained from hospital. In order to have a more thorough and accurate knowledge of H1N1 virus infection, a larger community-based research efforts are needed. Co-infection with other viruses could be present along with the H1N1 virus in the same patients who were included in this study.

CONCLUSION(S)

A greater prevalence rate of H1N1 infection was noted in this article. This illness particularly affects children under the age of 10. After analysing the data from this study, it was shown that reducing proinflammatory cytokines could be a proactive step to reduce IAV-related mortality and morbidity in the near future. IL3 levels were elevated by H1N1 infection, and this substance may be crucial for osteoclastogenesis in those with URTI s, particularly H1N1. Further studies will benefit greatly from an investigation into the function of other cytokines and chemokines as well as the vast spectrum of clinical diagnoses of all IAV and IBV lineages.

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