Original Article

DETERMINATION OF SPECTRUM OF HAEMATOLOGICAL PARAMETERS IN H1N1 POSITIVE INFLUENZA CASES ADMITTED IN A TERTIARY CARE HOSPITAL IN MULTAN

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ABSTRACT

Objective: To determine derangements of Haematological parameters in H1N1 positive influenza cases.

Material and Methods: It was a cross sectional study conducted in Department of Haematology, Combined Military Hospital Multan, from Nov 2018 to Jan 2019. All suspected cases with clinical features of influenza flu, who were admitted in Combined Military Hospital Multan from Nov 2018 to Jan 2019 were investigated for haematological parameters in Influenza A/H1N1. Multiplex PCR for influenza virus was conducted. Haemoglobin (Hb), Platelets count and Total leucocyte count (TLC)were performed in all suspected cases. A total of 221 cases were analyzed.

Results: Out of 221 cases that have been reviewed, 104(47.1%) were positive for influenza A/H1N1 and 117 (52.9%) had negative results for influenza virus. Out of 104, 73(70.2%) showed abnormal platelets count and 31(29.8%) had normal platelets count. However, 91(87.5%) showed no effect on TLC count but 13(12.5%) had raised TLC count and 90(86.5%) had normal haemoglobin level but 14(13.5%) had low Hb.

Conclusion: Platelets count decreased significantly during H1N1 infection, whereas TLC count increased but to lesser extent. However, haemoglobin did not show any significant change.

Key Words: H1N1 influenza, PCR, Hb, Platelets count, TLC count.

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INTRODUCTION

Influenza is an acute common viral infection previously known as swine flu. Seasonal influenza represents a year-round disease burden which usually peaks in winter months (December to February) [1,2]. It causes illness with varied presentation from fever, cough and headache to complications such as bronchitis, secondary pneumonia and even death [3]. In 2009, Influenza A (H1N1) pdm09 caused recent influenza pandemic of 21 century with high mortality rates in human with a death toll of about 284500 worldwide [4]. After the pandemic, A/H1N1pdm09 replaced the pre-existing seasonal influenza, a virus subtype H3N2 and influenza B lineages that was active for three decades [5]. It has high transmissibility, short incubation time and high rate of morbidity and mortality [6]. There are very limited no of studies on influenza virus related disease and morbidity in Pakistan.

Influenza A (H1N1) pdm09 belongs to orthomyxoviridae family, contains eight negative-sense, single-stranded ribonucleic acid (RNA) segment virus [7]. Influenza virus has subtypes on the basis of surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA). It usually targets the respiratory system but reports regarding involvement of other organ systems like liver, myocardium, kidney, gastrointestinal tract and skeletal system.

This is the study of its kind where we have used a basic test like blood complete picture which was commonly advised in cases admitted with suspicion of Influenza virus, a rare disease but epidemic can be a challenging task. This study will help in monitoring of such cases with these haematological variables, easily available in each setup.

MATERIAL AND METHODS

We carried out a cross sectional study in Combined Military Hospital Multan from Nov 2018 to February 2019. All patients who were admitted on the basis of suspicion of influenza were included in study and non-probability consecutive sampling technique was used for selection of patients. We excluded all known cases of anemia, pancytopenia and on any iron supplement from our study. This study was conducted on 212 patients, population contained both

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male and female, from the age group of 02 years to 80 years with suspicion of viral influenza. Specimens of nasopharyngeal swab were collected and kept in virus transport media. Real time Polymerase Chain Reaction (RT-PCR) for pandemic H1N1 virus was carried out on instrument ABI 7500, using one step quantitative RT-PCR probe hydrolysis Taqman kits supplied by National Institute of Health. Influenza A sub-tying primer and probe sets are designed to specifically detect contemporary Human A/H1 and A/H3 Inflluenza viruses. For a test to be valid, positive and negative controls were run before specimen. All patients, both positive and negative on the basis of Real time Polymerase chain reaction, were subjected on blood complete picture variables most specifically Haemoglobin level, Total leukocyte count and Platelet count to check a relation between H1N1 positive cases and H1N1 negative cases. Specimen for blood complete picture was collected in EDTA tube and samples were analyzed on Sysmex KX-21 Haematology analyzer. Quality control of haematology variables were carried out by using two level of controls, low level and normal level of EIGHTCHECK-3WP ASSAY. Kits used for Sysmex KX-21 include stromatolyser-WH and cell pack manufactured by Singapore. The results with low platelets count were rechecked by slide visual methods. Results of blood CP were compared in H1N1 positive and negative PCR analyzed groups.

Statistical Analyses: All the data were analyzed using SPSS version 24.0. Descriptive analyses was carried out after confirming the Gaussian nature of the data by Kolmogorov smirnov's, a Test of normality. Mean and SD was calculated for quantitative variables like haemoglobin, platelets count and total leukocyte count. One-Sample T Test was applied to compare mean of variables in H1N1 PCR positive and PCR negative cases. P value < 0.05 was considered as significant.

RESULTS

Out of the total 221 patients included in our study, 104 (47.1%) were PCR positive for H1N1 and 117(52.9%) were negative for influenza virus. Out of the total 221 cases, we had 59(26.7%) female patients and 162 (73.3%) male. Mean age of male and female patients were 34±12.04 years and 40±16.15 years respectively. When we compiled the results of 221 cases, mean value for Haemoglobin, TLC and Platelets count was 13.2±1.68 g/dl, 7.23 ± 2.88 $x10^{9/L}$, 229 ± 737 $x10^{9/L}$ respectively (Table-1). Out of 104 PCR positive H1N1 cases, 73(70.2%) showed low platelet count and 31(29.8%) had normal platelets (p value was statistically significant p=0.001). However, 91(87.5%) had no effect on total leucocyte count but 13(12.5%) had raised TLC level than the reference range, (p value was statistically significant p=0.003). It was further noted that in H1N1 PCR positive cases, 90(86.5%) had normal haemoglobin level but 14(13.5%) had low haemoglobin level (p value was statistically significant p=0.003). Kolmogorov Smirnov's, a Test of normality showed p < 0.001. To compare the mean value of haemoglobin, total leucocyte count and platelet count of PCR positive and PCR negative, One-Sample T Test was applied which showed p value 0.000 (Table-2). Figure-1 shows that platelets counts were prominently decreased in majority of influenza A/H1N1 cases while lesser effects were observed on TLC and Hb.

Table -1: Mean ± SD of Haematological parameters in all suspicious cases (n=221).

Variable	n	mean	SD(±)
Haemoglobin (g/dl)	221	13.2	1.68
TLC ($x10^{9/L}$)	221	7.23	2.88
Platelets ($x10^{9/L}$)	221	229	737

Table-2: Comparison between mean values of Haematological variables in Influenza A/H1N1 PCR positive and PCR negative cases by one-sample t test.

Variable	PCR +ve (n=104) Mean ± sd	PCR –ve (n=117) Mean ± sd	p value (<0.05)
Hb (g/dl)	13.1±1.8	13.7±1.5	0.003
TLC (x $10^{9/L}$)	6.9±2.8	7.6±2.9	0.003
Platelets($x10^{9/L}$)	226±688	233±780	0.001

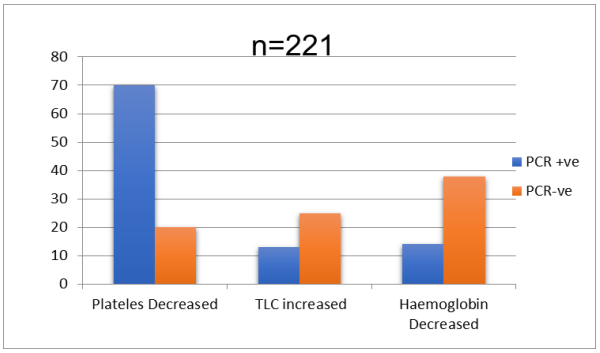

Figure-1: The graph shows significant decrease in platelets count in PCR positive cases as compared to other variables(n=221)

DISCUSSION

Viral influenza A/H1N1 is significant cause of mortality and morbidity and if undiagnosed, would affect quality of life in a developing country like ours. The diagnosis involves a battery of tests often including sophisticated tests like Polymerase chain reaction [8]. Outbreaks occur mostly in winter season and are always a challenging task to differentiate

between H1N1 influenza or seasonal flu. Multiplex RT-PCR assay has been considered as an important tool for diagnosis of different strains of influenza but has always remained a time taking process and cost has been a big challenge in our set up. We selected patients who presented with various symptoms like fever, headache, body aches, cough, sneezing and sore throat, admitted at Combined Military Hospital Multan from November 2018 to February 2019 with strong suspicion of Influenza A/H1N1 and their specimens were sent for PCR. This study has analyzed the results of blood complete picture which was commonly advised in these patients to determine any association of haemoglobin, total leucocyte count and platelets count in Influenza A/H1N1 PCR positive and negative cases. Two hundred twenty-one patient's specimen were sent for PCR, 104 (47.1%) were PCR positive for H1N1 which correlates with a study done in Nepal [9].

Our study found that significant number of patients with influenza A/ H1N1 had decreased in platelets count as compared to influenza negative/ common cold cases. The same results were found in a study by Elena Popa and Florin Rusu who noted that H1N1 influenza can lower the number of platelets [10]. It was further noted that effect of influenza A /H1N1 on total leucocyte count was less remarkable in positive cases for H1N1. This finding is in accordance with an article by Nianye Wang of China [11]. Similarly, Kashatriya, et al conducted a study in India, has results similar to our study regarding the effects on total leucocyte count[12]. However, no effects were found on blood haemoglobin variable which is also in accordance with earlier studies

CONCLUSION

Platelets count decreased significantly during H1N1 infection, where as TLC count and Haemoglobin level showed a lesser of change.

AUTHORS CONTRIBUTION

Hamid Iqbal: Idea conception.

Muhammad Younas: Literature review.

Syed Naeem Raza Hamdani: Manuscript proof reading.

Oshaque Ali: Data collection + statistical analysis.

Waqas Hanif: Result compilation.

Muhammad Aamir: Scientific inquiry and data collection.

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