



Prevalence of Cytopenia and Evaluation of Its Clinical Significance in Children with influenza A(H1N1)

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ABSTRACT

Aim: Although influenza A causes flu epidemics every year, in 2009 the virus named influenza A H1N1 caused a pandemic. Mortality rates were reported higher than expected due to secondary infections. The aim of this study is to evaluate cytopenia which is one of the hematologic complications in patients who present with flu symptoms and are positive for influenza A H1N1.

Method: 503 patients presenting at our Pediatrics Clinic with flu symptoms in November and December 2009 were retrospectively analyzed for epidemiologic and hematologic findings. The diagnosis of H1N1 was made with antigen tests studied on the nasopharyngeal swab.

Result: 269 cases were male and 234 were female. Mean age of the patients was 8.8 years (six months- 17 years). 149 of 503 cases (29.6%) were positive for influenza A and 354 cases (70.4%) were negative. Sixty-three (42%) of positive cases underwent full blood count. Nine (14.3%) of these had leucopenia, 12 (19%) had neutropenia and 10 (15.9%) had lymphopenia. Hemogram was performed on 120 cases who were negative for influenza A. Only three (2.5%) of these had leucopenia, 2 cases (1.7%) had neutropenia and 1 case (0.8%) had lymphopenia. The difference between the groups was statistically significant ($p<0.004$, $p<0.001$, $p<0.001$). None of the cases had anemia or thrombocytopenia. Cytopenia resolved in the follow-up with the disappearance of symptoms and there was no permanent morbidity in any of the cases.

Conclusion: Influenza A H1N1 infection leads to leucopenia but does not cause permanent cytopenia.

Key words: Influenza A, H1N1, cytopenia, leucopenia, neutropenia

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İnfluenza A (H1N1) Geçiren Çocuk Hastalarda Sitopeni Sıklığı Ve Klinik Öneminin Değerlendirilmesi

Amaç: İnfluenza A her yıl grip salgınlarına yol açmakta ise de, 2009 yılında influenza A H1N1 adlı virus pandemiye neden oldu. Bu salgında mortalite oranının gelişen sekonder enfeksiyonlara bağlı olarak beklenenden daha yüksek olduğu bildirildi. Bu çalışma grip semptomları ile baş vuran influenza A H1N1 pozitif olgularda hematolojik komplikasyonlardan sitopenilerin değerlendirilmesi amacı ile yapıldı.

Metod: Kasım ve Aralık 2009 da Hastanemiz Çocuk Hastalıkları Polikliniğine grip semptomları ile başvuran 503 olgunun epidemiyolojik ve hematolojik bulguları retrospektif olarak incelendi. Olgulara H1N1 tanısı nazofarinks sürüntüsünden çalışılan antijen testleri ile konuldu.

Bulgular: 503 olgunun 269 erkek, 234 kadındı. Hastaların yaş ortalaması 8,8 yıl idi (altı ay-17 yaş arasında). Beşyüzüç olgunun 149'unda (% 29.6) influenza A testi pozitif, 354 (%70.4) olguda ise influenza A testi negatif olarak saptandı. Testleri pozitif çıkan olgulardan 63'ünde (% 42) tam kan sayımı yapılmıştı. Bunlardan dokuz vakada (% 14,3) lökopeni, 12 vakada (% 19) nötro-peni ve 10 vakada (% 15.9) lenfopeni saptandı. İnfluenza A negatif olgulardan 120 tanesinde hemogram bakılmıştı. Bunlardan da sadece üç olguda (% 2.5) lökopeni, iki vakada (% 1.7) nötro-peni ve bir vakada (% 0.8) lenfopeni saptandı. Gruplar arasındaki fark istatistiksel olarak anlamlıydı ($p<0.004$, $p<0.001$, $p<0.001$). Olguların hiç birinde anemi ve trombositopeni gözlenmedi. Takiplerde semptomların kaybolmasıyla birlikte sitopenide düzelme görüldü ve hiçbir olguda kalıcı morbidite gelişmedi.

Sonuç: İnfluenza A H1N1 enfeksiyonu lökopeni gelişmesine yol açmakta , ancak kalıcı sitopeni yapmamaktadır.

Anahtar kelimeler: İnfluenza A, H1N1, sitopeni, lökopeni, nötro-peni

INTRODUCTION

Unlike previous seasonal flu, the new pandemic influenza A virus (H1N1) was seen to affect the young population mostly (1-3). The definite diagnosis of the disease is made with reverse transcription-polymerase chain reaction (rt-PCR) positivity (4). Children under 5 years are particularly at risk, and complications are more severe in children under 2 years (5,6). The most important risk factors for development of H1N1 infection are chronic lung disease, immunosuppression, cardiac disease, pregnancy, diabetes mellitus and obesity (7). Viral infections are known to cause hematologic complications particularly cytopenies. There are many published articles about the hematologic complications caused by viruses (8). However, there is limited information on the prevalence and course of cytopenia during the courses of the new pandemic influenza A (H1N1) infection (9,10).

Cytopenia was the most remarkable finding in the cases presenting with flu symptoms at our clinic. Therefore, we planned this study to investigate the prevalence of cytopenia which is a hematologic complication in the children positive for influenza A (H1N1) infection.

MATERIALS AND METHODS

Hemograms and influenza A antigen test results of 503 patients presenting with flu symptoms at the Pediatrics Outpatient Clinic of Private Sema Hospital in November-December of 2009 were retrospectively evaluated. Epidemiologic, clinical, laboratory and prognostic data

at presentation were obtained from computer records. Full blood count of influenza patients was carried out using HORIBA ABX Pentra 80 France unit. A total of five parameters including leucocytes and leucocyte subgroups (lymphocytes, neutrophils), erythrocytes and platelets were evaluated. The diagnosis of influenza A (H1N1) infection was made with rapid antigen tests (Flu A+B/ Rapid antigen tests, country) on the nasopharyngeal swabs.

Cytopenia was determined with decreases in the three main cell groups which are platelets, leucocytes and erythrocytes. Decreases in leucocytes and leucocyte subgroups (lymphocytes, granulocytes) were also analyzed. A platelet count below 150,000/mm³ was evaluated as thrombocytopenia in all age groups. In children under 2 years, a leucocyte count below 6,000/mm³ and in children between 2-17 years, a leucocyte count below 4,000/mm³ were considered as leucopenia. In children under 2 years, a lymphocyte count below 1,800/mm³ and in children between 2-17 years, a lymphocyte count below 1,000/mm³ were considered as lymphopenia; in children under 2 years, a neutrophil count below 1,100/mm³ and in children between 2-17 years, a neutrophil count below 1,500/mm³ were considered as neutropenia. Hemoglobin values below 10.5 g/dL in children under 2 years, below 11.5 g/dL in children between 2-9 years, below 12.5 g/dL in boys between 10-17 years and below 12.9 g/dL in girls between 10-17 years were considered as anemia (11) after ruling out underlying iron deficiency and vitamin B12 deficiency anemias; likewise, hematocrite levels below 32%, 33%, 35% and 36%,

Table. Cytopenia rates and p values in groups positive and negative for influenza

Hematologic complications	Influenza positive n (%)	Influenza negative n (%)	Total n (%)	p value
Leucopenia	9 (14.3)	3 (2.5)	12 (6.6)	<0.01
Lymphopenia	10 (15.9)	1 (0.8)	11 (6)	<0.001
Neutropenia	12 (19)	2 (1.7)	14 (7.7)	<0.001

respectively, were considered as anemia.

Peripheral smear assessment: A cytopenia giemsa stain (classical method) and peripheral blood smears were done for each patient. The results were then assessed by a hematology specialist. Leukopenia was observed in patients with atypical lymphocytes (larger cytoplasm with rare eosinophilic granules and normal lymphocytes that are 1.5 to 2 times larger than expected). The peripheral smear report stated that lymphocytes were observed. In addition, the leukopenia in the blood count results were consistent with leukopenia peripheral smear results. It was reported that atypical blastic specialized cells were not observed.

The complete blood count parameters of patients positive for H1N1 infection were compared with those negative for H1N1 by using chi square test and $p \leq 0.05$ was considered as significant.

RESULTS

Two hundred sixty nine (53.47%) patients were male and 234 (46.53%), female. The mean age of the patients was 8.75 years (range: 6 months-17 years). Of these 503 children tested for H1N1 infection, 149 cases (29.6%) were positive for influenza A (H1N1) infection and 354 (70.4%) were negative. Among 149 children positive for influenza A (H1N1) infection, 129 (86.6%) were treated at the out-patient clinic and 20 (13.4%) were hospitalized for the treatment.

Sixty three (42%) influenza A positive cases underwent complete blood count. Nine (14.3%) of these had leucopenia, 12 (19%) had neutropenia, and 10 (15.9%) had lymphopenia. One hundred and twenty influenza A negative cases also underwent complete blood count. Only 3 (2.5%) of these had leucopenia, 2 (1.7%) had neutropenia and only 1 (0.8%) had lymphopenia. The differences between the H1N1 positive and negative groups was statistically significant ($p < 0.01$, $p < 0.001$, $p < 0.001$,

respectively). None of the cases had anemia or thrombocytopenia. All the patients with leucocytosis had secondary bacterial infections. Patients were questioned about rheumatic diseases like cyclic neutropenia and SLE which cause chronic neutropenia and about malignancies and drugs. None of the cases developed anemia or thrombocytopenia. During the 4-week follow-up period, blood counts returned to normal together with the improvement of clinical symptoms. None of the cases had permanent cytopenia.

DISCUSSION

Influenza A (H1N1) cases were first seen in pigs and the first human case was reported in 1974 (12). Epidemics and deaths due to this virus started to be reported in the following years (13). In 2009, influenza A (H1N1) virus became effective as a pandemic. The definite diagnosis of influenza A (H1N1) is made with PCR. Sensitivity of the rapid antigen test in the pandemic influenza A (H1N1) virus infection is 10-70% compared with PCR. The specificity of this test is above 95%. It is reported that 99% of the influenza A virus causing a pandemic in 2009 was H1N1 (14-16).

There are many reports stating that in the course of viral infections, cytopenias particularly leucopenia and thrombocytopenia may develop due to autoimmune inhibition and/or inhibition at the level of precursor cells in the bone marrow. Apart from viral agents like hepatitis B and C viruses which cause marked cytopenia, there are also viral infections causing severe cytopenia like parvovirus B19 and HIV (17). Leucopenia is known to occur frequently in the course of influenza A infections, but isolated thrombocytopenia, anemia and pancytopenia are rare. There are few reports stating that the new influenza A (H1N1) may cause cytopenias. In a Chinese study on 426 cases, lymphopenia was found at a rate of 68% in adults while this was 92% in children (18). In another study on influenza A (H1N1) infection,

lymphopenia was found at a rate of 21% (18). In a study on 272 cases treated by hospitalization of the patients, leucopenia was noted in 20%, anemia in 37%, and thrombocytopenia in 13% of those cases (20). In another study on 553 cases, data of 30 patients treated by hospitalization were analyzed and 1 of 7 children aged 17 years or younger and without any underlying disease had anemia and another had lymphopenia while one child had leucocytosis accompanying lymphopenia (21). In a study by Chien et al (22) on 96 cases, the rate of leucopenia was 22% in the group developing respiratory failure. Grasselli et al (23) reported that they have noted lymphopenia in the first defined case in their country. Similarly in our study (503 cases), lymphopenia was found at a rate of 14.3% while anemia and thrombocytopenia was absent. Unlike other studies, we evaluated neutrophils as a subgroup of leucocytes and neutropenia was found at a rate of 19%.

It is reported that leucopenia, anemia and thrombocytopenia seen in the course of influenza A (H1N1) infection have improved with the resolution of viral infection symptoms and not cause permanent cytopenia and related serious complications (24). In the 4-week follow-up period, leucopenia improved in all cases together with the resolution of symptoms related to infection. None of the cases developed serious neutropenia or permanent neutropenia. None of our patients died.

In conclusion, it can be said that influenza A (H1N1) infection leads to a mild leucopenia but does not cause serious cytopenia. Absence of thrombocytopenia and anemia in our cases may be due to the small number of cases and absence of chronic diseases. It was noted that cytopenia resolved with improvement of the clinical picture and did not cause any morbidity in the follow-up.

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