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• Javan S, Tabesh M. Action of carbon dioxide on pulmonary vasoconstriction. J Appl Physiol. In press 2005

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ORIGINAL ARTICLE

Cognitive, Emotional, and Behavioral Problems of Children with Hemophilia

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ABSTRACT

Background: Children with hemophilia are prone to a variety of psychological problems due to some limitations associated with the disease. We aimed to compare the cognitive, emotional, and behavioral problems of children with hemophilia to healthy children.
Methods: This study was performed on 65 children with hemophilia and 65 healthy individuals as the control group who were between the ages of 7 and 12 years in Children's Hospital. The Child Behavior Checklist (CBCL) was used to identify emotional/behavioral problems and Wisconsin Card Sorting Test (WCST) to evaluate cognitive problems.
Results: The results showed that children with hemophilia obtained lower scores in activity, academic performance, and overall competence variables. Children with hemophilia in comparison to healthy children showed more internalizing and externalizing problems and emotional and behavioral deficits. Also they demonstrated more impairment in executive functions than healthy children.
Conclusion: The bio-psycho-social factors such as factors associated with the disease (e.g. anemia and bleeding), and the treatment (e.g. side effects of the drugs) and environmental and social factors are among underlying causes of some psychological problems in children with hemophilia.

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Introduction

Hemophilia is an inherited bleeding disorder linked to the X chromosome resulting in a genetic deficiency in clotting factor VIII (hemophilia A) or factor IX (hemophilia B). It is classified as mild, moderate, or severe considering the percentage of the factor that is produced.[1] Patients with hemophilia are at increased risk of joint bleeding (typically in the knees, elbows and ankles), intra peritoneal and intracranial hemorrhage, bleeding during and after surgery. There are also complications due to natural course of the chronic disease and the treatments the patients have to receive such as pain, anxiety, and depression.[2] Children with hemophilia encompass a difficult life since they have to deal with chronic arthropathy followed by fatigue and limb limitations. One of the serious concerns of parents and health care systems is emotional and behavioral problems in children with hemophilia which gets more complicated with increasing age and may affect their quality of life.[3]

Chronic diseases can lead to emotional, behavioral, and cognitive problems.[4] Children with chronic diseases confront extreme stress, deprivation and serious limitations associated with their disease.[5] Perceived stress can interfere with brain functions and may result in emotional and behavioral dysfunction. Hanson and colleagues indicated that difficult life circumstances in childhood can decrease hippocampus and amygdala volume which cause behavioral disorders.[6] Children with hemophilia are often deprived of regular life activities because of the fear of recurring episodes of bleeding.[7] The protracted stress can make them vulnerable to the brain atrophy which may result in behavioral problems.[8] Prolonged bleeding causes a feeling of fatigue and weakness in children with hemophilia. The vitality of

children will be reduced and it will provide a context for developing a sense of anger and frustration.[9] Children with hemophilia are susceptible to various infectious diseases and viruses such as hepatitis and AIDS.[10] Parents of these children show overprotective behavior towards their children, resulting in anxiety and chronic depression in the children.[11] Numerous studies have emphasized on low compliance of young patients with hemophilia to medical instructions. The emotional and behavioral problems disrupt the compliance with the treatment.[12]

Psychological consequences of hemophilia are worth studying. In this study, we aimed to assess the psychological status of children with hemophilia in three behavioral, emotional and cognitive aspects. Assessment of behavioral disorders and cognitive impairment in children with hemophilia by Wisconsin Card Sorting Test (WCST) is being investigated for the first time in our study comparing with healthy children.

Materials and Methods

The present research was a descriptive causal-comparative study. The study population consisted of all boys with hemophilia (aged 7-12 years) who referred to Children's Hospital. Healthy children were selected as the control group from siblings of the patients. The convenience sampling method was applied for the study. The exclusion criteria were as follows: 1) existence of comorbidity, 2) history of psychiatric disorders, 3) having the ability to participate in psychological interventions, and 4) presence of any kind of intracranial hemorrhage. The effect of gender variable was also controlled due to differences in incidence of behavioral and emotional disorders between girls and boys.

The sample consisted of 65 healthy children and 65 children with hemophilia who were assessed through Child Behavior Checklist (CBCL) and WCST. Informed consent was taken from the parents in advance explaining the motives of the study.

Child Behavior Check List (CBCL)

The Child Behavior Checklist (CBCL) is a report provided by the caregiver about children and is widely used in research and clinical practice. Generally, CBCL consists of the several following parts: 1) Academic performance assessment of children and adolescents in the fields of cognitive ability, training, and education issues, 2) Social skills assessment of children and adolescents for evaluating their adaptation with peers, siblings, parents and how they cope with challenges, and 3) Assessment of emotional and behavioral problems in children and adolescents.

The internal consistency of the CBCL was estimated using Cronbach's alpha coefficients. Alpha coefficient of competence was relatively high and its range was between 65- 85% for CBCL. The test reliability based on Achenbach experimental assessment approach and by using Cronbach's alpha was 0.89 for boys and 0.94 for girls and by using split-half reliability was 0.84 for boys and 0.87 for girls. The results of the construct validity supported the 8-factor structure of this scale by using factor analysis in Iran. In addition, convergent validity of this scale with "Junior Eysenck Personality Questionnaire (JEPQ)" and "Rutter behavioral problems questionnaire" was satisfactory.

If the index of discrimination power which is related to the variance and distribution scores reach higher than 90%, the scale will be appropriate. Obtained coefficients in the CBCL form were all at the high level. Based on the mentioned information about validity, it can be concluded that, CBCL is a valid tool in assessing behavioral and emotional problems and can be used with confidence by users.[13]

Wisconsin Card Sorting Test (WCST)

The Wisconsin Card Sorting Test (WCST) which was first developed by Berg and Grant is a useful tool for studying cognitive deficits after brain injuries.[14] WCST has been widely used as a neuropsychological test of "set-shifting", i.e. the ability to display cognitive flexibility and abstract reasoning. Participants must maintain a concept found at the stage of testing in sequential conditions. When the classification rules change, they must change the previous concepts. Subjects were given a set of 64 cards which there were 1 to 4 symbols on them as triangles, stars and circles in 4 colors: red, green, yellow, and blue. Of course, there were no two identical cards. Four cards including a "red triangle", two "green stars", three "yellow Plus sign" and four "blue circles" were used as the main cards. The participant was told to match the cards based on 4 main patterns on the cards. After each response, participants received right or wrong feedback. In fact, whether a particular match is right or wrong. Scores obtained from this test included: number of incorrect responses, perseverative errors, and percentiles of achieved categories. Validity of this test has been reported 0.86 for measurement of cognitive deficits after brain injury.

The Kolmogorov-Smirnov test was applied to test normal distribution. It indicated normal distribution of Externalizing/Conduct, Inattention/Hyperactivity, Internalizing/Emotional and Social/Peer variables in experience-based scales. There was no normal distribution in other variables. Therefore, independent T-test was used to analyze the findings of mentioned variables and other variables were analyzed by The Mann-Whitney test.

Results

In this study, children with hemophilia (n=65) were compared to healthy children (n=65) in terms of cognitive, emotional, and behavioral problems (Table 1). The analysis of covariance demonstrated that there were no significant differences in age and socioeconomic status between the two groups. The minimum and maximum age in children with hemophilia was 7.5 to11.2 years and in control group it was was 7.8 to 11.6 years. Most of the children in both groups were in third and fourth grade of elementary school. Most parents were educated at the university level. The severity of hemophilia in most patients were of moderate type.

As shown in Table 2, there was no significant difference

Table 1: Demographic data of children with hemophilia and healthy children

Variable		Children with Hemophilia M	Healthy children (siblings) M	%
Age of the child (min, max)		7.5,11.2	7.8,11.6	
School grade				
	1th			0.5
	2th			2.1
	3th			43.6
	4th			41.2
	5th			12.6
Mother's education	Diploma and under			23
	University graduated			77
Father's education	Diploma and under			35
	University graduated			65
Type of hemophilia				
	Mild			12.7
	Moderate			69.4
	Sever			18.6

Table 2: Mann-Whitney test to compare the behavioral-emotional problems in CBCL

Variables	Children with Hemophilia		Healthy children (siblings)		Mann-Whitney U	P value
	M	SD	M	SD		
Social functions	6.11	1.72	6.54	2.21	671	0.43
Academic Achievement	1.31	4.68	5.55	0.79	455	0.002
Anxiety/Depression	4.4	6.67	3.05	2.96	361	0.001
Withdrawal/Depression	2.67	3.33	1.57	1.18	369	0.001
Somatic complaints	2.05	2.17	1.3	0.92	472	0.004
Thought problems	3.83	4.23	1.91	1.38	355	0.001
Attention deficit	4.65	6.1	3.16	3.53	464	0.003
Aggressive behavior	8.93	11.47	4.72	5.03	372	0.001
Internalization	7.61	12.17	5	4.88	321	0.001
Affective disorder	3.77	4.87	1.62	1.79	321	0.001
Anxiety disorder	2.45	3.41	1.54	1.7	398	0.001
Somatization	1.27	0.61	0.46	0.88	722	0.721
ADHD	4.15	5.26	3.12	2.97	524	0.024
Oppositional defiant disorder (ODD)	2.31	2.43	0.98	1.22	482	0.005

between the two groups in average score of social behavior (P=0.430). The average score of academic performance in children with hemophilia was significantly lower than the healthy control group (P<0.01). Average scores of children with hemophilia in anxiety/depression, withdrawal/depression, somatic complaints, social problems, thought problems, attention problems, rule-breaking behavior, aggressive behavior, internalizing problems, emotional problems, anxiety, attention deficit hyperactivity disorder, and oppositional behavior were significantly higher than healthy children, while there was no significant difference between the two groups in physical problems (P=0.727).

According to Table 3, the result of t-test indicated that the average scores of activities and general competence variables in hemophilic children were significantly lower than control group and externalization and behavioral-emotional problems were significantly higher in children with hemophilia than healthy children (P>0.01).

As shown in Table 4, the average scores of the percentiles of achieved categories in children with hemophilia was significantly lower and preservation error variable was higher than healthy children.

According to Table 4, t-test results indicated that the average score of incorrect responses in children with hemophilia were significantly higher than healthy children (P>0.01).

Discussion

Based on the findings in our study, children with hemophilia indicated significantly lower scores than the control group in externalization/conduct, inattention/hyperactivity, internalization/emotions, and academic performance. This results were in line to the study of Coppola and colleagues who showed the academic achievement in children and adolescents with hemophilia were significantly lower than healthy children.[15] Children with hemophilia would be more absent from the school due to occasional bleeding episodes. On the other hand, physical limitations prevent them from participating in school activities and do their homework same as their healthy peers. This could explain their poor academic achievement.

Table 3: T-test to compare activities, general competence, externalizing and general problems according to CBCL form

Problem Scales	Children with Hemophilia		Healthy children (siblings)		t	P value
	M	SD	M	SD		
Externalizing/Conduct	16.24	5.13	6.28	2.18	4.93	0.001
Inattention/Hyperactivity	12.79	6.8	8.53	2.07	3.16	0.002
Internalizing/Emotional	17.6	4.09	11.62	2.72	3.34	0.001
Social/Peer	11.62	5.3	9.2	3.01	2.69	0.06
Total	49.73	13.18	20.93	7.22	5.13	0.001

Table 4: T-test of Wisconsin Card Sorting Test (WCST) for participants

Problem Scales	Children with Hemophilia		Healthy children (siblings)		t	P value
	M	SD	M	SD		
Categories Achieved	4.16	0.93	5.4	1.65	3.76	0.012
Failure to Maintain Set	3.67	2.5	5.17	4.06	4.15	0.001
Trial to first Category	2.36	2.91	5.32	3,45	4.97	0.001
Trial administered	15.8	4.31	19.2	2.43	2.43	0.03
Total trial Correct	16.1	3.55	20.43	2.64	2.67	0.01

The results of our study showed that social behaviors in children with hemophilia do not differ from healthy children. Chiu and colleagues investigated the social functioning of children with hemophilia; they showed there were no significant differences in popularity and social acceptance of children with hemophilia compared to their healthy classmates.[16] Chronic diseases cause increase in social sensitivity in the patients. They will also be concerned about judgment of their peers, so they comply with normal social behavior of their community to avoid negative self-image. As a result, they suppress their feelings and accept norms more easily.

Obviously, children with hemophilia compared with healthy children show more anxious/depressed and withdrawn/depressed conditions, somatic complaints, thought problems, attention problems and aggressive behavior, internalizing and externalizing problems and generally behavioral-emotional problems. Khair and colleagues conducted a qualitative study on 30 children with hemophilia.[17] They revealed that hemophilia had a significant effect on family lives, educational issues, school and traveling plans. Most of them felt frustrated and expressed anger against the disease. Tryzapch and colleagues also assessed emotional functioning in hemophiliac children.[18] As their reports, children with hemophilia showed more problems in internalizing and anxiety/depression disorders. Children with hemophilia showed symptoms of depression more commonly compared to healthy children.[19] There was also higher level of anxiety in hemophiliac children which was significantly correlated with the parents' attitude.[20] Children with hemophilia in comparison to healthy group displayed more affection issues, attention deficit hyperactivity disorder and oppositional defiant disorder. A four-year longitudinal study indicated that children with hemophilia experienced more emotional problems; however, their quality of life improved over the time.[21]

Current research findings indicate a cognitive impairment in children with hemophilia through Wisconsin Card Sorting Test (WCST). WCST as a neuropsychological test displays frontal lobe functioning and cognitive flexibility (for assessment of executive functions, behavioral regulation, and social discourse). In a systematic review, Janual and colleagues demonstrated that hemophilia could not cause per se cognitive deficit and reduced IQ, but adverse effects of the disease could lead to serious cognitive and behavioral damages.[22] So, we consider the bio-psycho-social factors as the main reason for behavioral and emotional problems.

Experience of pain and anemia effect on mood and temper of children with hemophilia (as bio factor). Over-parenting, lack of social learning and negative self-perception are involved in emotional problems (as a psychological factor). Children with hemophilia are less prone to socialization because they are afraid of participating in group activities. They are at increased risk for rejection by peers. So, social isolation may be a trigger for depression and anxiety (as a social factor). On the other hand, the child may feel anger followed by environmental and social deprivations. It would be expressed as aggressive behavior and conduct disorder.

In the study of Tryzapch and colleagues, children with hemophilia had not more externalizing problems compared to healthy children.[23] However, in this study, externalizing problems in hemophiliac children were more observed than healthy children. This difference may be explained by cultural and social issues, parenting styles and parents' exaggerated understanding of child behavior problems.

The results of our study showed children with hemophilia demonstrate more impairment in executive functions. Although, previous studies have shown children with hemophilia who had a history of intracranial hemorrhage experienced impaired intelligence and visual perception, there was no cases of intracranial hemorrhage in our study. We showed cognitive function is influenced by the disease itself even in the absence of an intracranial hemorrhage.

Restriction in physical and social activities of hemophiliac children from early childhood may interfere with development of cognitive processes. Also, many cognitive skills do not shape in these children properly.

Frequent absence from the school may be another factor that makes children to be less involved in school activities and it provides an inappropriate context for stimulation and training of intelligence and cognitive skills. This research suggests to perform future investigation of various psychological and neurological aspects in children with hemophilia in larger populations of the patients.

Conclusion

The bio-psycho-social factors such as factors associated with the disease (e.g. anemia and bleeding), and the treatment (e.g. side effects of the drugs) and environmental and social factors are among underlying causes of some psychological problems in children with hemophilia.

Conflict of Interest: None declared.

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Serum Levels of Glial Fibrillaryacidic Protein in Meningioma

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ABSTRACT

Background: Glial fibrillaryacidic protein (GFAP), an intermediate filament protein, is mainly expressed by astrocytes, but some other cells like enteric glia and non-myelinating Schwann cells can also express GFAP. GFAP elevation has been reported in some types of meningioma and malignant brain tumors. In the present study, we analyzed the association between serum levels of GFAP with meningioma.
Methods: Sixty-eight newly diagnosed patients with meningioma and 28 healthy individuals (control group) were included. Serum levels of GFAP were measured by ELISA.
Results: There was no significant difference in GFAP serum levels between the two groups. Subdivision of the patients also revealed no significant association between GFAP and meningioma.
Conclusion: We studied serum levels of GFAP in meningioma in Iranian patients for the first time. We did not observe a significant association between meningioma and GFAP. A larger study including a larger number of different subtypes of meningioma patients may discover a weakly significant difference if it exists.

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Introduction

Meningiomas are defined as tumors derived from arachnoid cap or meningothelial cells. They comprise approximately 35% of primary central nervous system (CNS) tumors according to the Central Brain Tumor Registry of the United States (CBTRUS) and thus they are considered the most frequently diagnosed primary CNS tumors.[1] Meningiomas are classified into three groups based on the WHO classification system:[2] In grade I, all tumors are benign including meningothelial, fibroblastic, lymphoplasmacyte-rich, transitional, angiomatous, microcystic, secretory, psammomatous, and metaplastic subtypes. Grade II tumors consist of atypical, clear cell, and chordoidmeningiomas. Grade III tumors are anaplastic, papillary, and rhabdoidmeningiomas. Grade II and III meningiomas are significantly more likely to have invasive disease with 30-50, and 50-94% rate of recurrence, respectively.[3,4] Brain-invasiveness in grade II or III tumors is histologically characterized by "irregular, tongue-like protrusions of tumor cells infiltrating underlying parenchyma without an intervening layer of leptomeninges". These changes are accompanied by reactive astrocytosis in the adjacent brain tissue.[5]

Glial fibrillaryacidic protein (GFAP), an intermediate filament protein, is mainly expressed by astrocytes but some other cells, such as enteric glia,[6] non-myelinating Schwann cells,[7] and human fibroblasts[8] in different tissues (i.e. meninges), can also produce GFAP. GFAP is involved in the structure and activities of cytoskeleton, mechanical support of the plasma membrane and maintenance of the shape of the cells.

GFAP expression is increased in injuries to the CNS with reactive gliosis, a process causing an increase in the production of intermediate filaments (GFAP,

vimentin, and nestin). Examples of these injuries are cerebrovascular trauma, stab wounds, and animal models of multiple sclerosis.[9] Moreover, in Alexander disease, a lethal rare neurological disorder, the astrocytes contain unique cytoplasmic inclusions that contain GFAP.[10,11]

Several studies have shown increased level of serum GFAP in astroglial tumors (i.e. astrocytoma and glioblastoma multiforme).[12-14] It was proposed as a glioblastoma multiform diagnostic marker in a study by Jung and colleagues in 2007.[15] Missler and co-workers demonstrated that GFAP level can be used as a serum marker of acute central nervous system damage in patients with traumatic brain injury (TBI).[16] Later, Voset and colleagues[17] and Nyle´n and colleagues[18] studied the use of serum level of GFAP as a prognostic factor in TBI.

Although GFAP is a main part of glial intermediate filaments, its immune-reactivity in meningioma was observed in prior studies.[19-25] A study evaluated serum GFAP level as well as some other immunohistochemical markers (e.g. epithelial membrane antigen and collagen IV) for the histological assessment of brain-invasive growth in meningioma. This study showed that stained sections in human meningioma are adequate for evaluating brain invasion and other immunohistochemical markers did not play significant roles in this issue.[26]

In this study we investigated the serum level of GFAP marker in the sera of patients with meningioma of different grades and also normal subjects. Our aims were to evaluate GFAP protein as a marker of meningioma tumor, and its possible association with the tumor grading.

Materials and Methods

Sera specimens were collected prospectively from 68 patients diagnosed with meningioma of different grades who had been admitted to Chamran or Nemazee hospitals in Shiraz, prior to any treatment. Their medical records were reviewed for pathology and some other characterizations of the tumor at the admitted hospitals. 68 cases of meningioma consisted of 41 cases of grade I, 7 cases of grade II, 8 cases of grade III and 12 cases with unknown grading. Control samples were obtained from 19 healthy people with no neurological complaints or known disease that had referred to Shiraz branch of Iranian blood transfusion organization. All of them provided informed consent prior to the study participation.

All sera were stored at -80 °C at the biobank of Shiraz institute for cancer research. Serum GFAP levels were calculated blind to the clinical data using a GFAP ELISA kit.

Data were analyzed using SPSS software, version 19. The Chi-square exact test was used and when the expected cell frequency was less than five, the test was replaced by Fischer's exact test. $P<0.05$ was considered as statistically significant.

Results

In the present study, we investigated serum levels of GFAP in 68 patients with meningioma and compared them to those in 19 healthy controls. The mean serum level of GFAP in patients with meningioma were 2.5±7.2 ng/ml (range: 0-46.122). The mean serum level of GFAP in the control group was 0.427±1.7 ng/ml (range: 0-7.482). Because GFAP serum levels were not normally distributed, the analysis was done by non-parametric Mann-Whitney U test which revealed no significant difference between patients and controls (table 1).

Serum GFAP level was detectable in 21 (30%) out of 68 meningioma patients, and 3 (15.7%) out of 19 people from the control group. The chi-square test also showed no significant difference in the number of patients with a positive value compared to the number of the controls with a positive value of GFAP (P=0.193).

Tumor grading was available in 56 of the cases, of which, 41, 7, and 8 had WHO grades I, II, and III, respectively. As it is indicated in table 2, grading of the tumor was not associated with GFAP serum levels (Kruskal–Wallis test, P=0.12). In grades I to III, 11, 5, and 2 patients had detectable values of GFAP, respectively. The number of the patients with a positive value was not significantly different according to the tumor grade as calculated using the chi-square test (P=0.15).

The location of meningiomas with positive serum GFAP were as follows: 5 tumors in sphenoid wing, 4 as a frontal mass, 3 tumor in falxcerebri, 2 in parasagittal area, 2 in posterior fossa, 2 convex meningioma, 2 in tuberculum sellae and another tumor with unmentioned site in the archived file. No significant association was also found between GFAP serum levels and tumor site.

Discussion

GFAP as the main protein of intermediate filament network in mature astrocytes was first detected in the

Table 1: Serum levels of Glial fibrillary acidic protein in meningioma patients and controls

Group	N	Mean±SD (pg/ml)	P value
CXCR4 patients	68	2.585±7.2	0.15
Controls	19	684.1±123.7	

Table 2: Glial fibrillary acidic protein serum levels based on tumor grade in meningioma

	N	Mean±SD (pg/ml)	Std. Deviation	Minimum	Maximum
1	41	2.8±8.4	8.416315	0.000	46.122
2	7	1.3±2.0	2.021615	0.000	5.711
3	8	0.56238±1.2	1.225123	0.000	3.457
Total	56	2.3±7.2	7.277253	0.000	46.122

P value was 0.129 and calculated using The Kruskal–Wallis test.

plaques of multiple sclerosis patients studied by Enget and colleagues.[9] Different types of GFAP proteins alongside vimentin, nestin, and synemin (other members of intermediate filament network) have been found in different subsets of astrocytes.[27,28]

In 1995 and 1996, four laboratories produced GFAP knocked out mice, of which, three reported the same number of neurons and astrocytes in these mice compared to wild types.[29-31] However, Liedtke and co-workers detected some decreased myelination in certain part of the brain and some tissue architecture differences in optic nerve and spinal cord.[32] All four studies showed that even in the absence of GFAP, reactive gliosis could be induced and vimentin would still be expressed. To learn more about this process, Eliasson and colleagues conducted another study in GFAP and vimentin knocked out mice. They showed cytoplasmic intermediate filaments in reactive astrocytes were not formed. GFAP and vimentin deficiency also caused a decreased reactive gliosis, scar formation and vulnerability to ischemia.[33] On the other hand, some other studies have shown better regenerative potentials such as better synaptic regeneration in the hippocampus in the presence of GFAP.[34,35] A role for GFAP in regulation of vascular flow has also been proposed; as in a study the brain infarction volume was higher after transient occlusion of carotid artery in GFAP null mice.[36] GFAP is also involved in cell motility,[37] cell division,[38] synaptic formation,[39] and maintenance of myelination of the CNS.[40]

GFAP overexpression has also been studied. Messing and colleagues conducted a study that used a human GFAP transgene to increase GFAP expression in astrocytes. 15 to 20-fold aggregation of GFAP proteins higher than controls was lethal. This study proved that GFAP mutations were the major cause of Alexander disease.[41]

In the process of maturation of astrocytes, GFAP substitutes vimentin as the major intermediate filament network protein and in some astrocytes vimentin expression decreases to undetectable amounts. GFAP expression continues to increase as aging occurs (probably as a consequence of aggregation of oxidative damaged proteins in the brain).[27,28] Rosengren and colleagues in 25 neurological healthy individuals detected an age-dependent increase in GFAP.[42] This change of GFAP serum levels was an indicator of changes in astrocyte functions as aging occurs.[42]

GFAP has been known as a diagnostic marker in glioma tumors. There has been some reports of positive serum GFAP in patients with meningioma in some previous studies that in most cases they had rhabdoid morphology or papillary variant (both type are aggressive). It was explained by a heterogeneous expression of GFAP in rhabdoid subtype of meningioma. Another possibility is that human fibroblasts in the meninges may express GFAP.[43] Two other studies have described a 'whorling-sclerosing' histological variant that the cases were positive for serum GFAP.[22,23]

In our study, some of the patients were positive for serum GFAP levels; however, it did not significantly differ from the control group. There was an atypical meningioma with focal rhabdoid feature that was not positive for serum GFAP level. We also had two cases of papillary meningioma who were negative for serum GFAP. No meningioma with "whorling-sclerosing" pathology was present in our cases.

There are some limitations to our study that requires more research. First our sample size was limited. Second, a number of the archived files were incomplete in some tumor features like tumor volume, staging, site, and type of the tumor that made more evaluation impossible.

Conclusion

We investigated serum levels of GFAP in patients with meningioma and compared them to those in the healthy control group. The mean serum level of GFAP in patients with meningioma compared to those in the control group was increased but did not reach a statistical significance. A larger study including a larger number of different subtypes of meningioma patients will help to discover any possible association.

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ORIGINAL ARTICLE

The Role of Bone Marrow Aspiration and Bone Marrow Biopsy in Diagnosis of Bone Marrow Metastases

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ABSTRACT

Background: Bone marrow is the site of many malignant disorders and it is one of the common places for solid tumors to metastasize. Examination of the bone marrow aspirate and biopsy is a routine procedure performed for assessment of various conditions such as cytopenias, hematologic neoplasms, nonmalignant disorders and metastatic neoplasms.

Methods: The patients were referred to the Hematology Department at Tishreen University Hospital. 236 patients enrolled the study. Both bone marrow aspiration and biopsy were performed for all patients. Bone marrow aspirate was interpreted by the hematologist and the biopsy was examined by a Histopathologist. Moreover, we used immunohistochemical staining of some bone marrow biopsy specimens in cases where more information for diagnosis is required.

Results: Bone marrow metastases was diagnosed in 35 (14.83%) samples. Prostate, breast, stomach, lung and neuromuscular cancers were metastasized to bone marrow in 11, 9, 7, 6 and 2 cases, respectively. Bone marrow biopsy could discover the metastasis in 100% of the involved cases, while only 40% of the cases with bone marrow involvement were diagnosed by bone marrow aspiration. The degree of sensitivity of bone marrow biopsy for diagnosis of bone marrow metastases in comparison to aspiration was statistically significant (P=0.001).

Conclusion: Bone marrow Metastases were diagnosed in 14.83% of the patients with malignant tumors. Prostate and breast cancer were the most common. Bone marrow biopsy could diagnose the metastases in all the cases compared to 40% by bone marrow aspiration.

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Introduction

Bone marrow (BM) is among the common sites for many malignant tumors to metastasize. A malignant metastatic tumor in BM usually means an incurable disease, although it is not necessarily fatal. Therefore, it is suggested to exclude BM involvement in any malignancy where the type of treatment is determined by stage of the tumor.[1] Examination of the BMA and BMB is a routine procedure performed for assessment of various conditions such as cytopenias, hematologic neoplasms, nonmalignant disorders and metastatic neoplasms.[2,3] BMB is an indispensable adjunct to the study of the blood disorders and may be the only method where the correct diagnosis can be made. BMA and BMB are easy and safe procedures and can be performed in outpatient clinics.[4] BMA is safer and easier than BMB which may be more associated with pain and bleeding.[5] BM is one of the most common places where metastatic transplants occur.

However, it is sometimes difficult to diagnose the presence of these metastases within BM for a variety of reasons and even BM involvement may remain undiagnosed until an advanced period of the disease.[6] Lung, breast, and prostate cancer do often metastasize to bone marrow, so bone marrow studies are essential in determining the tumor stage in these malignancies.[7] Diagnosis of BM metastases may also contribute significantly to the diagnosis of primary tumors.[8] As a result, the importance of using sensitive and specific screening methods to detect these metastases is highlighted. The histological and parenchymal study of the BM by BMA and BMB may give an idea of the primary tumor that caused metastasis if the primary tumor is unknown.[9] As a result, BMA and BMB are complementary in diagnosis.[10] The use of BMB is more important than BMA in the presence of BM fibrosis or infiltrations of tumor cells.[11,12] BMA has less sensitivity in the detection of solid malignant neoplasms and lymphoma compared with BMB.[13] BMB is the most reliable method of detecting the presence of infiltration within the BM.[14] BM is a preferred and frequent site for tumor metastases of several types such as breast, prostate and neuroblastoma.[14] In this study, we investigated the sensitivity and value of both BMA and BMB in the diagnosis of BM metastases.

Materials and Methods

236 patients were referred to the Hematology Department at Tishreen University Hospital during the period from Apr 2015 to Sep 2016. Most complaints in the medical records of the patients were isolated anemia or pancytopenia, general fatigue and weakness and a tumoral mass. Both BMA and BMB were performed for all patients. A series of laboratory and radiological examinations were performed as necessary and according to each case. BMA was interpreted by the hematologist and BMB by the Histopathologist. Moreover, we used immunohistochemical staining of bone marrow biopsy when necessary.

SPSS software version 22 was used for analysis. The Shapiro-Wilk test and Pearson correlation coefficient were used. The standard deviation of age was calculated in the study sample. Statistical significance was calculated at 95% confidence coefficient with statistical importance when the value of alpha was less than 0.05.

Results

The study sample consisted of 236 patients. The number of men was 142 (60.2%). BM metastasis was diagnosed in 35 (14.83%) patients. Prostate cancer was the most common tumor in 11 cases (Figure 1: A, B), breast cancer 9 cases, stomach cancer 7 cases, lung cancer 6 cases, and neuromuscular tumors in two cases (table 1).

Table 1: Distribution of solid tumors that showed a transition to bone marrow

Type	Number	Percentage
Prostate cancer	11	31.4%
Breast cancer	9	25.7%
Stomach cancer	7	20%
Lung cancer	6	17.2%
neuroblastoma	2	5.7
Total	35	100%

BM metastasis was diagnosed in all involved patients by BMB, while only 40% of the cases were diagnosed through BMA (Figure 2 A, B). The percentage of BM metastases diagnosed with lung cancer was 50% and the lowest was in the case of gastric cancer (28.6%). The degree of sensitivity of BMB to diagnosis metastasis in comparison to BMA was statistically significant (P=0.001). The results are shown in table 2.

In 9 cases, we could not confirm the presence of malignant cells (metastases) and it was necessary to conduct a bone marrow biopsy to confirm the diagnosis.

Discussion

The study was conducted to determine the importance of BMA and BMB and their usefulness in diagnosing BM metastasis. Infiltration of bone marrow by metastases is known to be common at advanced stages of some malignancies; hence BM could be a probable site for metastasis in solid tumors. BM metastases were often detected much more frequently than routine diagnostic

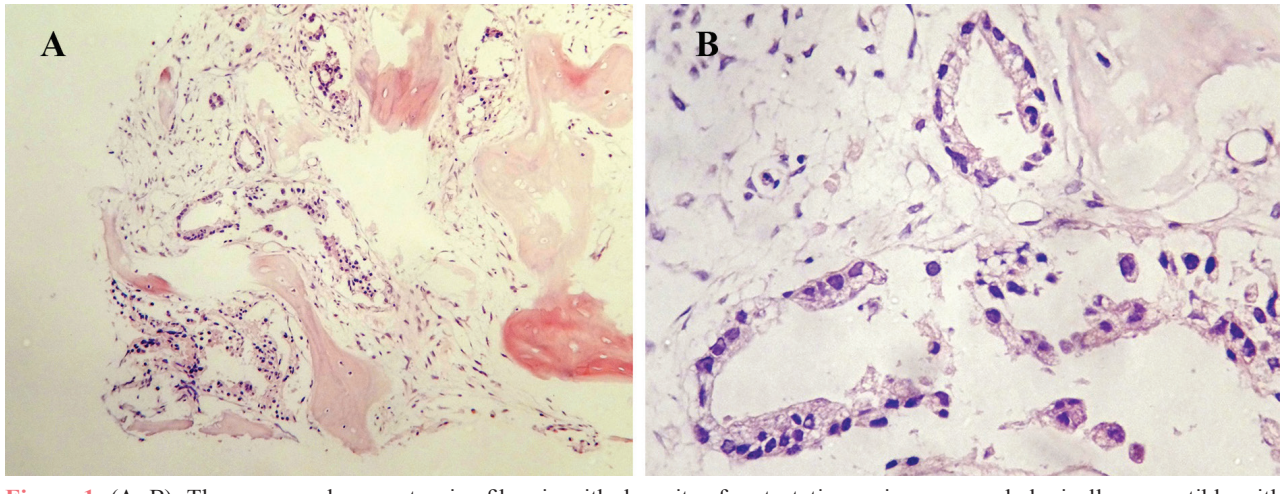

Figure 1: (A, B): The marrow shows extensive fibrosis with deposits of metastatic carcinoma morphologically compatible with metastatic prostatic carcinoma. Normal bone marrow elements are hard to identify.

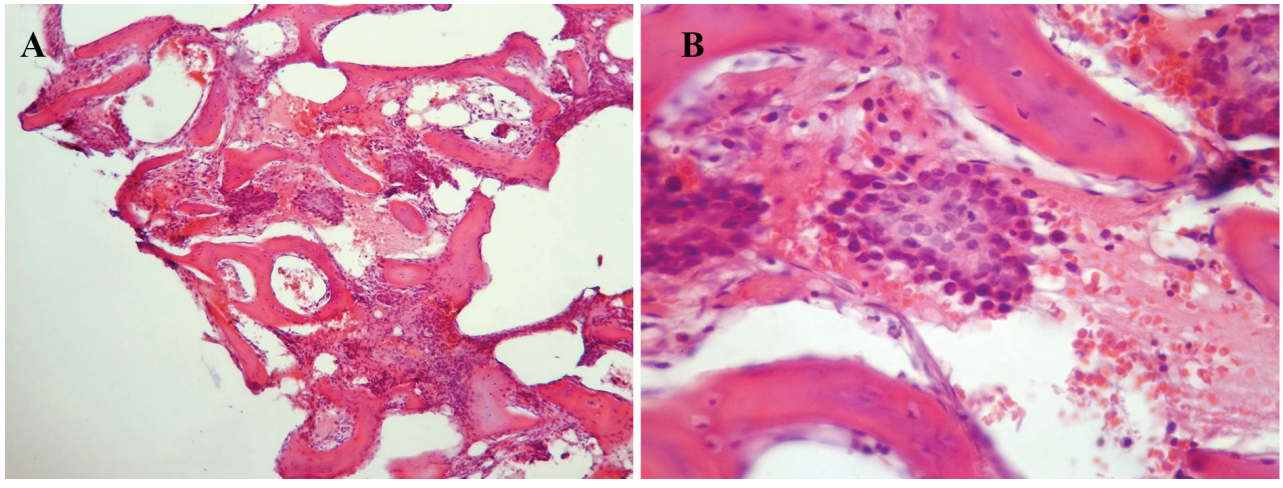

Figure 2: (A, B): Metastatic Carcinoma associated with marrow fibrosis. Normal Hematopoietic Elements are rarely seen.

Table 2: comparison between BMA and BMB in diagnosis of BM metastasis

Malignancy	No	BMB		BMA		P value
		No	Percent	No	Percent	
Prostate cancer	11	11	100%	4	36.6%	0.001
Breast cancer	9	9	100%	4	44.5%	
Stomach cancer	7	7	100%	2	28.6%	
Lung cancer	6	6	100%	3	50%	
neuroblastoma	2	2	100%	1	50%	
Total	35	35	100%	14	40%	

procedures.[7] Anemia was present in 65.7% of patients, thrombocytopenia in 14.3% and pancytopenia in 17% of the patients. However, there were directed signs and symptoms such as bone pain which was present in 20% of patients and hypercalcemia and high alkaline phosphatase in 22.9% of the cases and the abnormal laboratory tests was anemia with thrombocytopenia and sometimes pancytopenia with the overall hypercalcemia in some cases.[15] BMA is less sensitive to the detection of BM metastasis than BMB.[16] Diagnosis of metastasis in 14.8% of study patients makes it important to conduct a study to compare the sensitivity of BMA and BMB, as it may be thee ky to the diagnosis of primary solid tumors elsewhere in the body.[17] It may be noted that BMB may give an idea of the type of the tumor. This narrowed our search for the primary source of the tumors.[7] On the other hand, the study of bone marrow has little importance in detection of malignant lesions unless it is associated with other tests that may support the diagnosis such as pancytopenia.[18] Prostate, lung, and breast cancer are tumors that proliferate commonly within the BM.[19] There are also some less invasive tumors in bone marrow such as neuroblastoma, stomach and colorectal cancer.[7] The superiority of BMB in the diagnosis of BM metastasis makes it important to include it in workup of cases suspicious for BM involvement; however, BMA continues to play its role in the diagnosis or orientation of metastases. Because of the importance of bone marrow studies, combining both procedures of BMA and BMB increases the diagnostic yield of diagnosis of BM involvement [1]. All cases in which BMA was positive were associated with positive results in biopsy, which makes BMA highly sensitive to the diagnosis of BMM, but the value remains low compared to the BMB.

Conclusion

BM metastases were diagnosed in 14.83% of the patients. Prostate and breast cancer were the most common metastatic tumors and the stomach and lung cancer were in decreasing order. BMA could detect metastases in 40% of the involved subjects suggesting a superior role for BMB in the diagnosis of BM metastases. However, BMA continues to play a major role in the diagnosis of BM metastases. Due to importance of diagnosis of bone marrow involvement, combining BMA and BMB increases the diagnostic yield of diagnosis.

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Conflict of Interest: None declared.

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Comparative Effect of Chamomile Mouthwash and Topical Mouth Rinse in Prevention of Chemotherapy-Induced Oral Mucositis in Iranian Pediatric Patients with Acute Lymphoblastic Leukemia

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ABSTRACT

Background: Oral mucositis afflicts more than 3/4 of patients with cancer under chemotherapy. In acute cases it could lead to brain damage caused by hypoxia and even death due to airway obstruction and reduction of chemotherapy drug dose. We aimed to compare the effects of topical mouth rinse and chamomile mouthwash in prevention of oral mucositis caused by chemotherapy in children with cancer.

Methods: The study was a randomized double-blind clinical trial on 62 children aged 6-15 years with acute lymphoblastic leukemia under chemotherapy. The participants were divided randomly into two groups. The first group used topical mouth rinse and the second group started to use chamomile mouthwash a day before chemotherapy through 14 days. Mucous membrane status was assessed before starting the treatment (one day before chemotherapy), 7th and 14th day and it was reviewed based on WHO oral mucositis check list assessment and then registered by the researcher.

Results: The results showed that the frequency of severity of oral mucositis in both groups did not have any significant difference 7 days after chemotherapy (P=0.46). The severity of oral mucositis in those who had used chamomile mouthwash 14 days after chemotherapy was significantly lower than those who used topical mouth rinse (Z=3.23, P=0.001).

Conclusion: In short term, using chamomile mouthwash and topical mouth rinse to prevent oral mucositis is effective in children with cancer.
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Introduction

Survival of pediatric cancer patients has been dramatically increased as a result of multimodality approaches such as surgery, radiotherapy, and intensive chemotherapy.[1] In the United States, acute leukemia comprises about 27% of childhood cancers.[2] Systemic chemotherapy has been used to eradicate leukemic cells; various protocols with different cytotoxic potentials have been used for childhood ALL.[3]

IC-BFM 2002 protocol is prescribed for childhood ALL and has been used in children with ALL aged 1 to 18 years. This protocol was conducted in clinical trials since 2002 and today it is one of the most important protocols for treatment of childhood ALL.[4]

Most of the chemotherapeutic agents inhibit cellular proliferation and hence have unfavorable side effects on healthy tissues that proliferate rapidly such as bone marrow and gastrointestinal mucosa. Oral mucositis is

one of the main complications of chemotherapy which is debilitating in cancer patients and occurs commonly following chemotherapy or radiation therapy.[3]

Oral mucositis is defined as inflammatory changes which occur in buccal and labial mucosa, the inferior surface of the tongue, sublingual folds and soft palate. In early stages the first clinical sign is a white-milky layer which severe scarring develops after 1-2 weeks with loss of epithelial structure.[5] On the other hand, reduced doses of chemotherapy or any delay in treatment could cause serious problems and might increase mortality to 40% in patients undergoing chemotherapy.[6] In physiologic conditions, the normal oral mucosa and natural salivary activity are two important barriers to prevent the invasion of microorganisms. These barriers will be impaired by the occurrence of oral mucositis.[7] Among chemotherapeutic agents in childhood ALL, methotrexate and cytarabine cause bone marrow suppression and are commonly associated with oral and intestinal mucositis.[8] Mucositis usually develops 3-5 days after starting chemotherapy and reaches its peak after 7-14 days.[9] There is no standard approach for prevention and management of oral mucositis in children and all currently used approaches are still under survey in clinical trials. The most important factor to prevent damage to the oral mucosa is maintaining oral hygiene.[10]

German chamomile is one of the most widely used herbs in pharmaceutical products worldwide and chamomile mouthwash is produced from the extract of this plant.[11] This plant contains chamazulene, alpha bisabolol, bisabolol oxides, spiro ethers, and flavonoids which have anti-inflammatory, antibacterial and antifungal properties.[12]

Mazokopakis et al. has reported a case of methotrexate-induced oral mucositis in a patient with rheumatoid arthritis who was treated successfully with chamomile mouthwash.[13]

There are also other products such as local anesthetic agents, antibiotics, antiacids, nystatin and sucralfate which are used for relief of oral mucositis or the pain itself.[14] There is a report that allopurinol mouthwash has been able to relieve the severity of stomatitis and associated pain.[15]

In this study we aimed to assess the efficacy of topical mouth rinse with compounds of (sucralfate, allopurinol, bicarbonate 7.5% and serum half-saline) which has been used in this study and in return they have used mainly chamomile mouthwash to prevent mucositis resulted by chemotherapy.

Materials and Methods

Study Design

This study was a randomized, double-blind clinical trial which compared the effects of chamomile mouthwash with topical mouth. The powdered pill (sucralfate, allopurinol) combined with sodium bicarbonate 7.5% and half-saline serum were given to the test group as a topical mouthwash for prevention of oral mucositis elicited by chemotherapy in children with ALL. 62 children (31patients received topical mouth rinse and 31 patients received chamomile mouthwash treatment) aged 6-15 years old, admitted to 17 Shahrivar hospital of Rasht city were enrolled into this study. All the patients were receiving protocol 2002 BFM.

The content and methods of this study were approved by the Research Council and Research Ethics Committee (approval no: IR.MUI.REC.1394.4.38) of Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran, before initiation of data collection. After text review regarding the safety of chamomile mouthwash and topical mouth rinse and clarifying the research goals to children and their parents, all the parents signed a written informed consent before participation in the study. All the patients were informed that participation in the study is voluntary and were assured that their personal information would be kept confidentially. Researchers were committed to consider the participants' rights in accordance to the principles explained in the Helsinki Declaration.

One day prior to the start of the treatment with methotrexate and cytarabine, the questionnaire including the demographic data was completed by the investigator and the patients' mouth was assessed for the presence of any mucositis or ulcer based on the oral mucositis check list of WHO.

World Health Organization's Oral Mucositis Check List

In this scale, zero has been defined as lack of oral mucositis, grade 1 as sore and erythema, grade 2 as ulceration and erythema in the mouth while being able to eat solid food, grade 3 ulcer and extensive erythema in the mouth and ability to just drink liquids and grade 4 as mucositis to the extent that there is inability to eat or drink even liquids.

Instruments

Toothbrush and toothpastes were given to both groups and they were taught how to brush properly. Also, the precise way of mouthwash application (chamomile or topical mouth rinse) was taught and they were asked to record the frequency of mouthwash usage in a check list prepared by investigator in order to control and follow up.

The test group started to gargle the mouthwash a day before chemotherapy continued for 14 days, every day after brushing, three times a day (morning, afternoon, evening) and every time 20 cc (without any dilution) for a minute so that all parts of the mouth, gums and tongue be smeared. They didn't eat up for an hour after mouth rinsing and all other treatments were continued other than the consumption of chamomile.

The control group used chamomile mouthwash available in pharmacies (30 ml drop Matrika mouthwash barijessans/kashan, Iran) for mouth rinse since the day before chemotherapy for 14 days afterwards, every day after brushing, three times a day (morning, noon, night). Thus, they diluted 30 drops of solution in 20 cc water and then gargled for a minute so that all parts of mouth, gums and tongue smeared. They didn't eat up either for an hour after mouthwash and previous standard treatment of doctor continued other than topical mouth rinse consumption.

Data Collection/Procedure

Data were collected between July to December 2015. The study started since the day before chemotherapy until 14 days thereafter. The patient' mucosal status and existence of any kind or degree of mucositis in the mouth or throat were recorded according to executive protocol by the investigator before starting treatment (one day before chemotherapy) and then on seventh and fourteenth day based on oral mucositis check list assessment of WHO.

Data Analysis

Data were analyzed by statistical software of SPSS version 11, using descriptive statistics and chi-square test, T-test, Mann-Whitney-Wilcoxon tests.

Results

Evaluation of unit's distribution according to sex and age of divided groups is shown in Table 1. Chi-square test showed that the frequency of sex in both groups were not significantly different (χ^2=0.07, P=0.79) and independent t-test showed that there was not a significant difference between two groups in terms of mean age (t=0.28, P=0.78).

The Frequency of Oral Mucositis in Terms of Severity on Seventh and Fourteenth Day after Chemotherapy

On seventh days after chemotherapy, forty-one patients (66%) were free from oral mucositis, twelve patients (19%) showed grade 1, five patients (8%) grade 2, three patients (5%) grade 3 and one patient (2%) experienced grade 4 oral mucositis, whereas 14 days after chemotherapy, thirty-four patients (55%) were free of oral mucositis (grade 0), seventeen patients (27.5%) had grade 1, five patients (8%) grade 2, two patients (3%) grade 3 and four patients (6.5%) experienced grade 4 oral mucositis. (Figure 1)

The Frequency of Severity of Oral Mucositis in Two Groups on Seventh Days after Chemotherapy

The results of this study showed that the frequency and severity of oral mucositis in two groups, seven days after chemotherapy was not significantly different (P=0.46). In other words, in short term, (7 days) the effect of topical mouth rinse and chamomile mouthwash on oral mucositis caused by chemotherapy was not different.

The Frequency and Severity of Oral Mucositis in Two Groups on Fourteenth Days after Chemotherapy

The results showed that the frequency and severity of oral mucositis, 14 days after chemotherapy was significantly less in those who had used chamomile mouthwash than control group who had used topical mouth rinse. (Z=3.23, P=0.001, table 2).

Table 1: Distribution of sex and the mean age of the subjects in both groups

Personal records	Groups	Local mouthwash		Chamomile mouthwash	
		Number	Percentage	Number	Percentage
		Average	SD	Average	SD
Age		9.7	3.01	9.9	2.9
Sex	Boy	17	54.8	18	58/1
	Girl	14	45/2	13	41/9
Overall		31	100	31	100

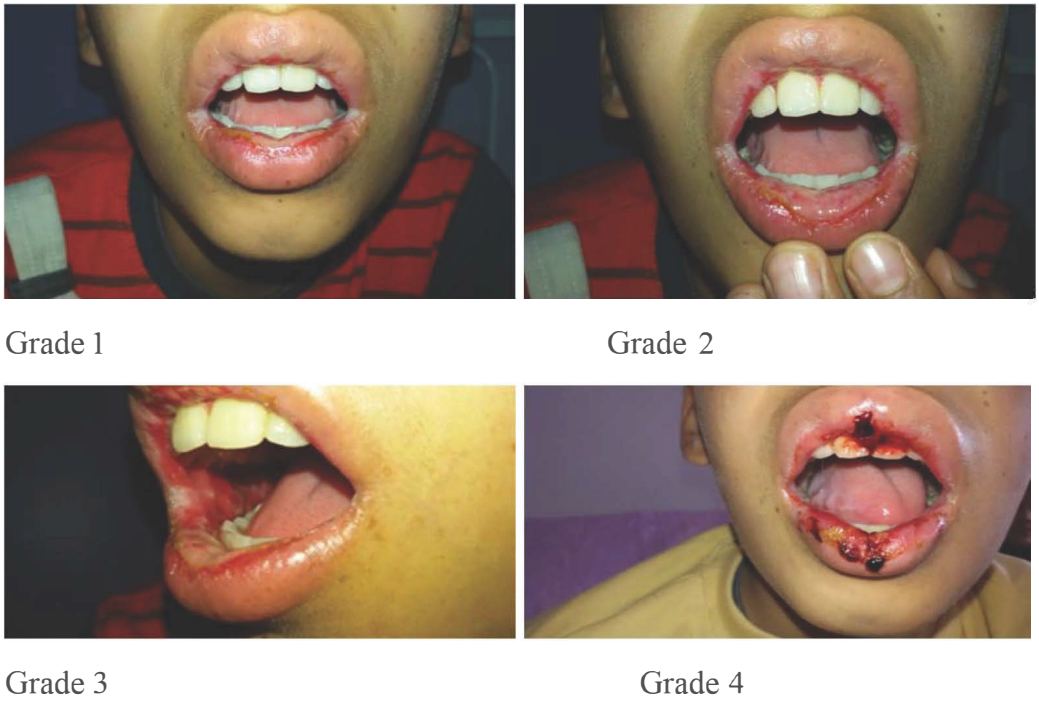

Figure 1: Oral mucositis and ulcers in different grades.

Table 2: Frequency distribution of oral mucositis in two groups, 7 and 14 days after chemotherapy

Mucositis severity	7 days after local mouthwash consumption		14 days after local mouthwash consumption		7 days after chamomile mouthwash consumption		14 days after chamomile mouthwash consumption	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Without mucositis	20	64.5	11	35.5	21	67.7%	23	74.2%
Grade 1	4	12.9	11	35.5	8	25.8%	6	19.2%
Grade 2	3	9.7	4	12.8	2	6.5%	1	3.3%
Grade 3	3	9.7	1	3.3	0	0	1	3.3%
Grade 4	1	3.2	4	12.8	0	0	0	0
Overall	31	100	31	100	31	100	31	100

Discussion

This study showed that the frequency and severity of oral mucositis in two groups, seven days after chemotherapy was not significantly different (P=0.46). However, in the previous studies reported from Iran,[16] They reported that there was a significant difference in number of patients with oral mucositis on the day 7, in group of patients who had used chamomile as mouthwash in comparison to the control group (P=0.01). According to the investigator, the observed difference in their study could be explained by comparing the chamomile mouthwash with a placebo (sterile water); while in our study chamomile mouthwash was compared to a topical mouth rinse comprised of sucralfate, allopurinol, bicarbonate 7.5% and serum half-saline. In our study, the severity of oral mucositis on seventh day after chemotherapy was lower in the chamomile mouthwash group; however, it was not significant.

The severity of oral mucositis, 14 days after chemotherapy, was significantly lower in group of patients who had used chamomile rather than topical mouth rinse. The results of our study in this longer period did not match with the previous study.[12] It seems that the difference could be due to the age of the subjects in this study who were children with ALL; aged 6-15 year, but in Fiedler's study; the average age of the participants was 64.3 years old. In addition, the type of chemotherapy could have some contributions since in Fielder's study it was 5-Fluorouracil, while it consisted of methotrexate and cytarabine in our study. Another study from Iran has reported lower rate of occurrence of oral mucositis on seventh day after chemotherapy in patients treated with chamomile mouthwash. (P=0.01). However, on 14th day of chemotherapy, the incidence of oral mucositis in patients treated with chamomile mouthwash was not significantly different with the placebo group [16] (P=0.5). In that study, the pathophysiology of the mucositis process was mentioned as the reason for the discrepancy. They also stated the symptoms and intensity of mucositis from fourth day and its subsidence almost after 2 weeks; while in the present study the mucositis severity on day 7 and 14 after chemotherapy in the group of patients using chamomile mouthwash had no significant difference based on statistical analysis. According to Adamson and colleagues, the most important side effect of methotrexate was bone marrow suppression with oral and intestinal mucositis. Meanwhile, the most adverse effect of cytarabine is reported to be bone marrow suppression with gastrointestinal mucosal injuries that occur between days 5-14 after treatment.[9]

Fiedler conducted a study to assess the efficacy of chamomile mouthwash on prevention of stomatitis caused by 5- Fluorouracil. It showed that chamomile mouthwash had no beneficial effect on incidence of oral mucositis induced by 5- Fluorouracil (P=0.32).[12]

The beneficial effect of chamomile mouthwash on oral mucositis caused by methotrexate has been reported in a patient with rheumatoid arthritis.[13] In our study, the severity of oral mucositis 14 days after chemotherapy, in the group who consumed topical mouth rinse, was significantly more than 7 days after chemotherapy [Z=2.05 (P=0.04)]. In other words, the severity of mucositis at the day 14 after chemotherapy was more than what was observed on 7th day of chemotherapy. In another study, the topical mouth rinse of various combinations failed to prevent the occurrence of oral mucositis in children with cancer.[8] In this research the topical mouth rinse had less effect in preventing mucositis compared to smectite cream glycerin, and also no serious adverse effects were observed in both groups.[8] Sucralfate is suggested as an effective topical mouth rinse. Its effect on the pain following tonsillectomy in children aged 6-12 has been investigated and has showed positive results.[17]

Allopurinol has also been proposed as a topical mouth rinse and it was one of the ingredients of our topical mouth rinse. The efficacy of allopurinol mouthwash on prevention of chemotherapy-induced oral mucositis has been studied.[15] It showed that allopurinol mouthwash could significantly decrease severity of oral mucositis and its associated pain.

Conclusion

Based on the results of this study, chamomile mouthwash in comparison to topical mouth rinse (sucralfate, allopurinol, bicarbonate 7.5% and serum half-saline) is an effective compound in prevention of oral mucositis in children with cancer.

Conflict of Interest: None declared.

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ORIGINAL ARTICLE

Use of Capillary Electrophoresis for Detection of Hemoglobinopathies in Individuals Referred to Health Centers in Masjed-Soleiman

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ABSTRACT

Background: Hemoglobinopathies are the commonest single gene disorder in human that affect hemoglobin production and function that occur when mutations alter the amino acid sequence of globin chains. The purpose of the present study was to evaluate the prevalence of hemoglobninopathies detected by capillary electrophoresis method in individuals referred to Masjed-Soleiman health centers by capillary electrophoresis method.
Methods: This study was carried out on 394 individuals referred to Masjed-Soleiman health centers during 2015-2016. Blood samples were collected in EDTA vacutainer tubes, then CBC including blood indexes (MCV, MCH), level of Hemoglobin A, Hb F, Hb A2 and other hemoglobins were evaluated by Sebia minicap (France) and also genetic tests applied for them to confirm results that were aqcuired by capillary electrophoresis method.
Results: 77 (19.5%) subjects had HbA2 ≥3.5%, thus were classified as beta thalassemia carrier and 3.3%, 2.5%, 1.5% and 0.5% of the individuals were heterozygote for Hb S, Hb D, Hb C and Hb Bart, respectively. Results of the genetic analysis showed the mutations in these subjects; cd36-37(-T) was the most frequent mutation in beta thalassemia carriers in this geographic region.
Conclusion: This study showed high frequency of beta thalassemia mutations in the geographic region of Masjed-Soleiman (19.5), and 7.85% of the individuals had hemoglobin variants including Hb S, Hb D and Hb C detected by capillary electrophoresis. Capillary electrophoresis could be a considerable method for detection of hemoglobinopathies.

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Introduction

Inherited hemoglobin disorders known as hemoglobinopathies are caused by mutations of the globin genes. Globin chain is made up of four polypeptide chains; these chains are of four types: α, β, δ, and g. Each molecule of hemoglobin consists of two pairs of unlike globin chains. The hemoglobin disorders fall into two main groups: the structural hemoglobin variants and the thalassemia. Single nucleotide substitutions can lead to hemoglobin variants or hemoglobinopathies. In normal adults, 96-98% of the Hb is Hb A (α2β2), with small amounts (23.5%) of HbA2 (α2δ2) and about 1.5 % of Hb F (α2g2).[1]

According to recent statistics, approximately 7% of the global population carries an inherited Hb disorder gene and about 500,000 infants are born with a severe hemoglobin disorder annually.[2] Currently, up to 1000 hemoglobin variants have been registered.[3] Some of the hemoglobin variants are common such as Hb S (the most common worldwide), Hb C, Hb E and Hb D-Punjab.[4]

Likewise, other hemoglobin variants such as D, S, C, Lepore, Setif, CS, Q, J and other hemoglobins have been reported in many countries including Iran.[5]

β-thalassemia is commonly observed in individuals of Mediterranean, African, and Southeast Asian ancestry. In Iran the gene frequency of ß-thalassemia mutations is high and varies from area to area. In southern Iran, the gene frequency is also high and is about 8-10%.[6] As a result, diagnosis of hemoglobin variants and thalassemia has become increasingly important in clinical laboratories. In agreement with the guidelines of the British Committee for Standards in Hematology, numerous techniques for the screening and diagnosis of hemoglobinopathies have been developed such as cellulose acetate electrophoresis (CAE), isoelectric focusing (IEF), low-pressure liquid chromatography (LPLC), high- performance liquid chromatography (HPLC), capillary zone electrophoresis (CZE) and finally genetic analysis.[7] Cellulose acetate method is a common method for detection of hemoglobinopathies; however, differentiation of Hb variants with low concentrations, especially unstable hemoglobin can be difficult.

HLPC is a useful method for screening and diagnosis of hemoglobinopathies, but interference of glycated Hb S and Hb E with HbA2 quantitation may result in incorrect diagnosis of beta thalassemia in the presence of glycated Hb S and Hb E.[8,9] In 2007, Food and Drug Administration (FDA) approved the Sebia Capillarys CZE system for the evaluation of hemoglobinopathies.[10]

Reliable evidence has indicated that CZE may be an accurate tool for the screening and diagnosis of hemoglobinopathies. You-Qiong and colleagues analyzed adult and cord blood sample of patients heterozygous for Hb New York by using both CZE and HPLC. Interestingly, all cases could be diagnosed with CZE, whereas none of them could be detected by HPLC.[11]

Masjed-Soleiman is located in southern Iran (Khuzestan province) and the Bakhtiar population is the prominent ethnic group in this area. The frequency of the consanguineous marriage is high in there. We aimed to evaluate the prevalence of hemoglobinopathies in this population by capillary electrophoresis.

Material and Methods

This study was carried out on 394 individuals (51 %men and 49% women) that were referred to Masjed-Soleiman health center during 2015-2016. Blood samples were collected in EDTA vacutainer tubes, then CBC including blood indexes (MCV, MCH) were performed and hemoglobin A, Hb F, Hb A2 and other hemoglobin variants were evaluated by capillary electrophoresis (CE).

CE was performed using the Minicap system according to manufacturer's guidelines. The instrument is equipped to re-suspend, lyse, separate, and analyze EDTA whole blood for hemoglobin variants. Samples were tracked using a built-in bar code reader and electropherograms were produced automatically. The lysed red cells are electrophoresed in alkaline buffer (pH 9.4) allowing separation to be directed by pH and endosmosis. Detection of eluting hemoglobin species is accomplished using the change in absorbance 415 nm. An electropherogram is divided into 15 zones that each zone is entitled as Z.[12] Then genetic analysis including, ARMS-PCR, RFLP-PCR and sanger sequencing applied for confirming the results of CE method.

Results

77 of 394 samples (19.5%) showed Hb A2 >3.5 %, thus were classified as beta thalassemia carrier state. 74(18.7%) of them had MCV<80.0 fL, MCH<27.0 pg, but 3(0.75%) had normal blood indexes (MCV, MCH). The genetic analysis revealed various mutations in minor beta thalassemia; the most frequent was cd 36-37(-T) (table 1).

There were individuals who were heterozygote for Hb S, Hb D, Hb C and Hb Bart while their blood indexes were in normal range (table 2). The genetic analysis for these variants showed mutations in the β-globin gene, HBB: c.20A>T), HBB: c.67G> C and HBB:c.19G>A in

Table 1: Blood Indexes and mean and standard deviation of hemoglobin variants

Indexes of blood	Hemoglobin variants	Mean percent ±SD	Frequency No (%)
MCV≥ 80,MCH≥ 27	Hb S	16.22±3.8	12 (3.3)
MCV≥ 80,MCH≥ 27	Hb D	35.57±13.7	10 (2.53)
MCV≥ 80,MCH≥ 27	Hb C	8.13±2.46	4 (1.52)
MCV<80,MCH< 27	Hb Bart	1.1±.49	2 (0.5)
Total			28 (7.85)

Table 2: Frequency of Mutations of Beta thalassemia

Type of mutations of beta thalassemia	Number	Frequency
cd 36-37(-T)	28	36.36%
IVSII-1 (G>A)	16	20.77%
IVSI-110(G>A)	11	14.28%
Cd 82-83(-G)	8	10.38%
5UTR+20(C>T)	6	7.79%
IVS II-745(C>G)	4	5.19%
cd82-83(-G)	3	3.89%
Fr 8.9	1	1.29
Total	77	100%

Hb S, Hb D and Hb C, respectively.

The subjects with Hb A2 < 3.5% could be suspected of having a thalassemia or iron deficiency or association of β thalassemia with iron deficiency.

Discussion

Khuzestan province is a province with high frequency of mutations for alpha and beta thalassemia and hemoglobinopathies C, S and D.[13] Hemoglobin D is a beta chain variant, observed mainly in northwest India, Pakistan and Iran (south, north, and west of Iran).[14] Hb D could be seen in combination with sickle hemoglobin and beta thalassemia. Co-inheritance of beta-thalassemia and Hb D together can result in the slightly lower hemoglobin levels.

Hb S results from a point mutation in beta globin chain gene. Sickle cell disease is very frequent in southern of Iran, especially in Khuzestan province that sickle cell trait is usually asymptomatic with normal RBC indexes.[15] On the other hand, measurement of Hb A2 is challenging because its level could be low and also interference with other hemoglobin variants would change the quantity of Hb A2 and since molecular techniques are not routinely used in many medical laboratories, so that the most unknown hemoglobin variants may not be correctly diagnosed.[16]

The aim of this study was screening of hemoglobin abnormalities by using of capillary zone electrophoresis. In this study 19.5% of the individuals were classified as beta thalassemia carriers. 3.3%, 2.5%, 1.5% and 0.5% of the subjects were heterozygote for Hb S, Hb D, Hb C and Hb batrs, respectively. The numbers approximately were similar to the study performed by Joshaghani and colleagues in North of Iran. In that study, Hb electrophoresis was carried out by capillary electrophoresis and 0.27%, 4.68%, 55%,0.27% and 0.41% were recorded for Hb E, Hb D, Hb S, Hb H and Hb Bart, respectively. In that study, Hb D had a higher frequency than our study.[17] We did not have any case of Hb E in our samples.

Zandian et al. studied frequency of alpha and beta thalassemia mutations and hemoglobin C, D, and S in Ahvaz. Hemoglobin S was the most frequent hemoglobinopathy that was similar to our study.[13]

In another study which was performed in Ahwaz, the frequency of alpha and beta thalassemia and other hemoglobinopathies was investigated. The Results of their study showed the frequency of Hb S, D, C, and α–globin gene mutations to be 16.2%, 3.2%, 1%, and 9.7%, respectively which again was similar to the present study.[18]

In the present study, we used capillary zone electrophoresis for detection of hemoglobinopathies. Recent studies have illustrated that capillary electrophoresis separates HbA2 well from Hb E, Hb C, and Hb S and is suitable for screening. Cellular acetate electrophoresis is routinely used in clinical laboratory; however, is not much accurate. On the other hand, HPLC method is costly and not routinely available. It can achieve simultaneous analysis, fast separation, good resolution, high accuracy, and full automation. Furthermore, capillary electrophoresis also is capable to separate Hb A2 from Hb Lepore than the HPLC method.[19]

kim et al. compared the capillary electrophoresis method with cellulose acetate method for screening of hemoglobinopathies. The study was performed in two groups, one group with normal CBC and the other group were subjects with hypochromia and microcytosis. No statistically significant difference was found for Hb quantification (P>0.05). The study indicated that capillary electrophoresis was more sensitive than cellulose acetate for detecting Hb fractions.[20]

Higgins and coworkers analyzed evaluation of Hb A2 in patients with and without beta-thalassemia, and assessed heterozygous patients for Hb E, Hb S, Hb C and Hb D Punjab by using of capillary system. The results of this study demonstrated that the capillary method is superior to the Variant II method for HbA2 quantified measurement.[21]

Weykamp et al. evaluated the analytical interference of Hb S, Hb C, Hb D, Hb E, Hb J and Hb G on Hb A1c accuracy and concluded that glycated Hb could be reliably measured with CZE.[22]

In another study, Pornprasert et al. developed specific quality control materials for analysis of some forms of thalassemia and Hb variants that are commonly observed in South-East Asia. Interestingly, the Hb typing control materials could be stored and then accurately analyzed by the many commercially available techniques, including HPLC and CZE, thus representing a valuable resource for internal and external quality assurance in the diagnosis of hemoglobinopathies.[23]

In another investigation by Wan Asmuni and coworkers, cord blood was used for Hb E screening through capillary electrophoresis. It showed that implementation of a screening strategy using capillary electrophoresis on cord blood samples in areas where Hb E hemoglobinopathy is prevalent, is highly recommended as it is feasible and the disorder would be detected earlier in life.[24]

Conclusion

This study showed high frequency of beta thalassemia (19.5%) and other hemoglobin variants including Hb S, Hb D and Hb C in Masjed-Soleiman region and also indicated that capillary electrophoresis could be a considerable method for detection of hemoglobinopathies.

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Ethical Approval

The Ethics Committee of Islamic Azad University, Masjed Soleiman Branch approved the study.

Conflict of Interest: None declared.

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Case Report

Macrophage Activation Syndrome as the First Presentation of Juvenile Idiopathic Arthritis

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ABSTRACT

Macrophage activation syndrome (MAS) is a rare feature of rheumatic disorders in children and adolescence and its presentation as the first symptom of rheumatic disorders is very infrequent.

A 9-year-old girl, in whom MAS developed, was admitted to our Hospital in Tehran, Iran. She suffered from high grade fever and rash followed by multiple joint swelling months afterwards. Bone marrow aspiration and biopsy showed normocellular marrow with a cellularity of 90%. Benign-looking macrophages were remarkably increased; many of them showed hemophagocytic features. According to the presentation of long-standing fever and observation of "hemophagocytic macrophage" in bone marrow, MAS was diagnosed for the patient. Additionally, due to recurrent joint swelling in following months, she was diagnosed to be affected by "Juvenile Idiopathic Arhtritis" complicated by MAS.

MAS is a rare complication of rheumatic disorders which should be considered as the first presentation of rheumatic disorders in children specifically in those presenting with high fever, hepatosplenomegaly, lymphadenopathy and severe cytopenia.

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Introduction

Macrophage activation syndrome (MAS) is a life-threatening complication of rheumatic disorders, particularly systemic Juvenile Idiopathic Arthritis (JIA).[1] It is associated with uncontrolled activation of T lymphocytes and macrophages.[2] This uncontrolled activation of immune system develops in a group of diseases including infectious, neoplastic and rheumatic disorders.[3] Patients may become acutely ill with non-remitting high fever, hepatomegaly, splenomegaly, lymphadenopathy, pancytopenia, liver disease, coagulopathies and neurologic symptoms.[4] Macrophage activation syndrome is a rare feature of rheumatic disorders in children and its presentation as the first symptom of rheumatic disorders is rarer. Clinicians usually use the Hemophagocytic lymphohistiocytosis (HLH) criteria for diagnosis of MAS in practice. HLH should be considered in differential diagnosis of every pediatric patient with fever, splenomegaly, lymphadenopathy and pancytopenia.[5] Herein, we describe a 9-year-old girl who was diagnosed with MAS as the first presentation of systemic JIA.

Case Presentation

A 9-year-old girl was admitted with high grade fever

(T>39°C) of one month duration and bicytopenia. Past medical history and family history was unremarkable. During her admission, she developed limb weakness with a predominance of upper limbs and skin rash on her left leg. The patient was constantly febrile with pallor and cervical lymphadenopathy. Later on, hepatosplenomegaly, maculopapular rash on left leg and multiple joint swelling and tenderness became evident as the main physical findings. The patient met the criteria of American College of Rheumatology for the classification of Juvenile Idiopathic Arthritis and the diagnosis was applied accordingly.[6]

Complete blood count showed white blood cell count 12,000/ L (neutrophil 78%; lymphocyte 17%; monocyte 4.7%), hemoglobin 8.4 g/dL, hematocrit 27.2%, and platelet count 84,000/ L. Liver function tests were increased with AST 80 IU/L (up to 40) and ALT 45 IU/L (up to 41), serum albumin 2.7 g/dL (normal: 3.8-5.4 g/dL), Alkaline Phosphatase 286 IU/L (up to 240) and LDH 1431 IU/L (207-414). Renal function tests were normal. Other laboratory results were as follows: sodium 130 mEq/L, total cholesterol 114 mg/dL, Triglyceride 136 mg/dL, and serum ferritin >3000 ng/mL (7-140). Erythrocyte sedimentation rate was 54 mm/hr and C-reactive protein was 72.2 mg/dL (up to 5). Coagulation tests and disseminated intravascular coagulation profiles showed prothrombin time 13.4 sec (normal control 12 sec), activated partial thromboplastin time 26 sec (normal control 25-35sec) and fibrinogen 668 mg/dL (180-530).

Serological tests for viral infections such as Epstein-Barr virus, cytomegalovirus and herpes simplex virus, Wright and Widal agglutination tests were all negative. There was no evidence of viral infections or hepatitis. A Blood culture positive for Acinetobacter was reported. Complement and immunoglobulin levels were as follow: C3 171 mg/dL, C4 23 mg/dL, CH-50 95 mg/dL, IgG 1866 mg/dL, IgA 253 mg/dL, IgM, 127 mg/dL. Antinuclear antibody (ANA) was negative.

Bone marrow aspiration and biopsy showed normocellular marrow with a cellularity of 90%. Granulocytic and megakaryocytic lineages were normal in maturation, but erythroid lineage was hypoplastic. Benign-looking macrophages were remarkably increased; many of them showed hemophagocytic features (figure 1). As a result, she was diagnosed as having MAS complicating JIA; in fact, MAS was the first presentation of the underlying rheumatologic disease in the patient.

Intravenous dexamethasone (4 mg/day) was administered followed by IVIG (10 g/day). However, his symptoms and clinical signs did not improve. Fever was sustained and abnormal laboratory findings such as pancytopenia and transaminitis was not corrected. Consequently, immunosuppressive therapy with methylprednisolone (2 mg/kg/day for three days which was switched to oral prednisolone) and cyclosporine (2.5 mg/kg/day) was started. On the two next days after treatment with cyclosporine, fever disappeared. After 5 days, cytopenia recovered to hemoglobin 10 g/dL, hematocrit 36.1%, and platelet count 110000/ L. Liver function tests also normalized with AST 12 IU/L, ALT 26 IU/L, and serum ferritin decreased to 1100 ng/mL.

The patient then was referred to the rheumatology department while she was receiving treatment with cyclosporine and prednisolone. Cyclosporine discontinued after one year. The patient is in remission for both conditions (JIA and MAS) on 5 mg prednisolone every other day.

Discussion

MAS and its association with JIA was first defined by Hadchouel et al. in 1985.[7] Stephan et al. proposed the term MAS in 1993.[8] Based on literature review; up to 2008, more than 100 MAS cases have been reported worldwide.[9] The mortality rate is reported to be about 8% to 22%.[10] Early recognition and immediate treatment play an important role in prognosis of this entity. However, because of the lack of the established formal and universally accepted criteria, diagnosis of MAS is often difficult and confusing. Clinicians usually use the HLH criteria for MAS in practice as mutually are heterogeneous diseases, despite the fact that both originate from histolytic disorder and are recognized as a subtype of HLH.[11]

Currently, MAS is widely recognized as a severe

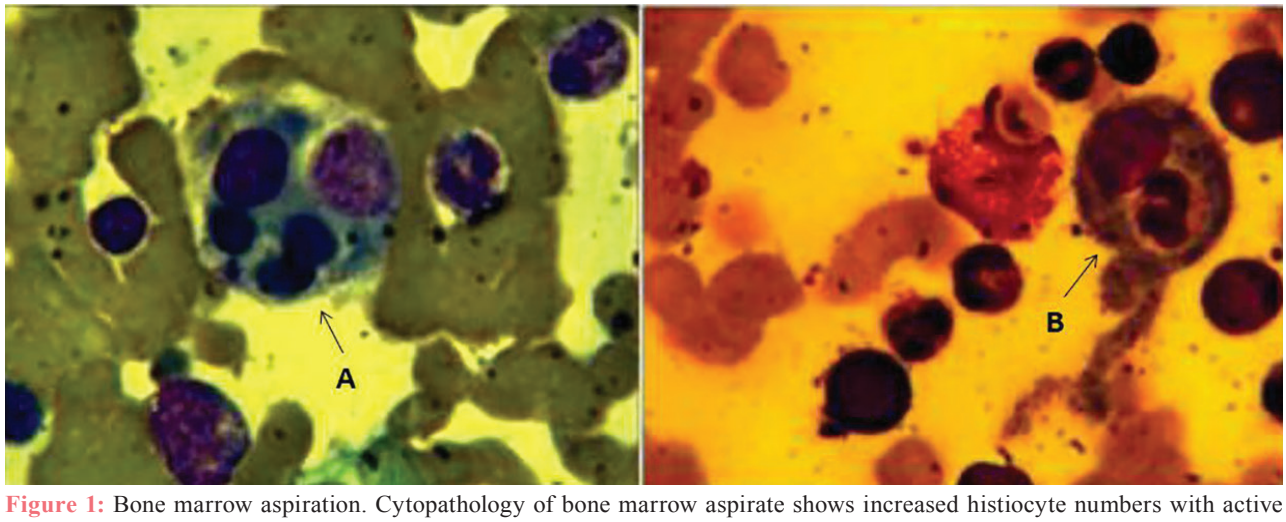

Figure 1: Bone marrow aspiration. Cytopathology of bone marrow aspirate shows increased histiocyte numbers with active hemophagocytosis (WrightGiemsa stain, ×400). **A**: A macrophage phagocytosing RBC. **B**: phagocytosis of neutrophil by macrophage.

and potentially fatal complication of JIA and has been commonly used to characterize the hemophagocytic syndrome that may develop in children with chronic rheumatic diseases specially systemic JIA.[7] MAS occurs during the clinical course of underlying Systemic JIA characterized by repetitive disease flares. Clinically, the pattern of fever and skin rash is not the same as JIA, although both entities share in common manifestations such as lymphadenopathy, hepatomegaly and splenomegaly.[1] Trigger factors may be drugs such as aspirin, nonsteroidal anti-inflammatory drugs, methotrexate and viral infections, specially Epstein-Barr virus and family of herpes viruses.[2] The diagnostic hallmark of MAS is hemophagocytosis in the bone marrow.[12] Our patient did not fulfill MAS criteria initially;[13] however, hemophagocytosis, which was found later in bone marrow aspirate was compatible with the diagnosis of MAS. The sensitivity and specificity of clinical and laboratory findings of MAS are defined.[14] The other variables that were in favor of MAS in our patient were serum ferritin ≥10,000 ng/mL, triglycerides ≥160 mg/dL, AST ≥40 IU/mL, ALT ≥40 IU/mL, gamma-glutamyl transferase ≥40 IU/mL and platelet count ≤150,000/L along with hepatomegaly and splenomegaly. Variables that did not prove sufficiently sensitive and specific included fever ≥38^0 c, lymphadenopathy, neurological manifestations, arthritis, rash, WBC ≤4,000/ L, ESR ≤50 mm/hr, LDH ≥ 900 IU/mL, bilirubin ≥1.2 mg/dL, fibrinogen 668 mg/dL and serum sodium of ≤130 mEq/L.

Hyperferritinemia is also a notable marker of MAS development making early and aggressive immunosuppression possible.[15] In our case, clinical and laboratory features of MAS improved dramatically after the initiation of immunosuppressive treatments.

MAS is a fulminant complication generally presenting in an acute and dramatic way. A review of the cases reported in the literature showed that MAS usually occurs during JIA treatment, but in our case it occurred as the presenting manifestation of JIA.

Clinical manifestations of MAS is occult and hard to diagnose in absence of clinical suspicion. In a recent cohort study to differentiate MAS in JIA from familial HLH and virus-associated HLH, a notable number of patients diagnosed with MAS showed values for white blood cells (84%), neutrophils (77%), platelets (26%), and fibrinogen (71%), which were within or above the normal range.[16] The exact incidence of MAS in childhood systemic inflammatory disorders is not entirely clear.[17] Moradinejad et al.[18] reported an incidence of MAS to be 8.2% in Still's disease. Although it generally develops in the early phase of JIA, it has been known to occur up to 14 years after diagnosis.

Triggers like infections or medications may precede the onset of MAS[1]. In our case, blood culture was positive for acinetobacter that might have been acted as a trigger.

JIA complicated by MAS is associated with significant morbidity and mortality. High-dose corticosteroid is the initial treatment in MAS and cyclosporine is used for severe or corticosteroid-resistant cases[(4)]. Currently, a standard treatment protocol for MAS is still lacking. Our experience in this case confirmed the efficacy of cyclosporine therapy. Results point out that the appropriate cyclosporine serum level during the onset of MAS should be as high as 200 - 300 ng/ml. Also IVIG may play a crucial role in the treatment of recurrent MAS.

Conclusion

Macrophage activation syndrome is a rare complication of rheumatic disorders in children and should be considered in patients presenting with non-remitting high fever, hepatosplenomegaly, lymphadenopathy, severe cytopenia and liver disease. Interestingly, MAS and HLH both could be considered as differential diagnosis for lymphadenopathy, splenomegaly, and cytopenia; however, MAS can be observed as the first symptom of JIA in children and adolescence. In uncertain cases, a bone marrow aspiration for identification of haemophagocytosis is suggested.

Conflict of Interest: None declared.

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Letter to Editor

A case of CML-like Disease with t(8;22)(q24;q11)

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Dear Editor

Chronic myelogenous leukemia (CML) is characterized in 85-90% of cases by the presence of the Philadelphia (Ph) chromosome and *BCR-ABL* fusion gene.[1] A further 5-10% of cases have other translocations, most commonly complex variants that involve one or more chromosomal regions in addition to bands 9q34 and 22q11, but also simple variants that typically involve 22q11 and a chromosome other than 9. There are a few reports regarding observation of t(8;22) in patients with CML-like disease.[2-6]

We report a case of CML-like disease with t(8;22) who achieved hematological remission with hydroxyurea and Imatinib. A 27-year-old Iranian male presented with fatigue and malaise. Physical examination revealed bilateral axillary lymphadenopathy and huge splenomegaly. Peripheral blood smear showed hyperleukocytosis with shift to the left, basophilia, and eosinophilia. Bone marrow aspiration and biopsy was in accordance with CML in chronic phase. Cytogenetic study revealed t(8; 22)(q24; q11) in all 20 metaphases analyzed. The BCR-ABL fusion was positive which was proved to be falsely positive due to BCR gene disruption. (Figure 1A).

Bone marrow FISH study using D-FISH probes were negative for the BCR-ABL fusion in 200 interphase cells analyzed for this patient (Figure 1B). By D-FISH, the metaphases showed red (ABL) signals on both copies of chromosome 9; one large green (BCR) signal on the normal chromosome 22 with smaller green signals on the der (22) and on the der (8). These findings were consistent with the known karyotype and suggested

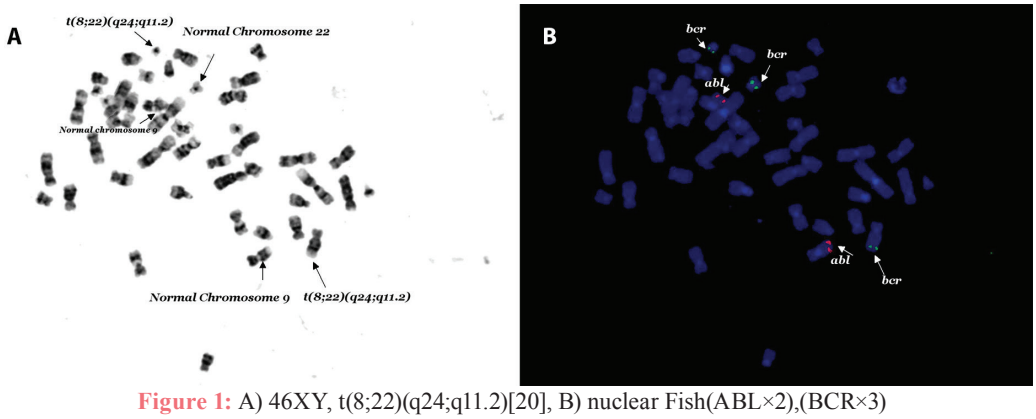

Figure 1: A) 46XY, t(8;22)(q24;q11.2)[20], B) nuclear Fish(ABL×2),(BCR×3)

that the chromosome 22 breakpoint must be close to, or within, the BCR. He initially received Imatinib mesylate and hydroxyurea which was followed by imatinib alone. He achieved complete hematological remission.

Although t(9;22) is diagnostic for CML, t(8;22) is another known cytogenetic abnormality in patients with CML-like disease. t (8;22) might have been classified cytogenetically as merely a simple variant of the t(9;22). A translocation between the long arms of chromosomes 8 and 22 described both in B-cell acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphomas (NHL), especially in Burkitt lymphoma has been also reported with BCR breakpoint in 22q11.2 in CML-like disease. CML-like disease with t (8; 22) can benefit from TKI therapy.

Conflict of Interest: None declared.

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PHOTO CLINIC

Solitary Plasmacytoma of the Humerus

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A 43-year-old man presented with pain in right arm since one year. A radiograph of the right arm showed an extensive osteolytic lesion involving the diaphysis of the humerus (figure 1). A biopsy and nailing was done. Histopathological examination showed sheets of plasma cells with few immature forms (figure 2). On immunohistochemistry, the tumor cells were CD138 positive with lambda light chain restriction, indicative of plasmacytoma. His hematology and serum chemistries were normal. His quantitative serum immunoglobulins and free kappa lambda were normal. Skeletal survey and bone marrow were normal. He received radiation 40 Gy to the humerus and is currently on follow up.

Solitary plasmacytoma of bone (SPB) is a localized tumor in the bone composed of a single clone of plasma cells in the absence of features of multiple myeloma such as anemia, hypercalcemia, renal insufficiency, or multiple lytic bone lesions. It constitutes about 5% of all plasma cell disorders.[1] The median age at diagnosis is 55 to 65 years and they present with skeletal pain or pathological fracture. SPB occurs more commonly in bones of the axial skeleton such as vertebra and skull.[2] Involvement of the appendicular skeleton is less frequent and humerus is a rare site for SPB. Diagnosis is confirmed by biopsy showing monoclonal plasma cell infiltration from a single site.

The treatment for SPB is local radiation therapy at a dose of 40-50 Gy. Surgery may be required for patients with structural instability of the bone, or rapidly progressive cord compression. The 10-year overall survival was 73% and local relapse free survival was 94%.[3] Overt multiple myeloma develops in 65-84% of patients in 10 years in spite of radiation therapy and the median time to progression is 2-3 years.[3,4]

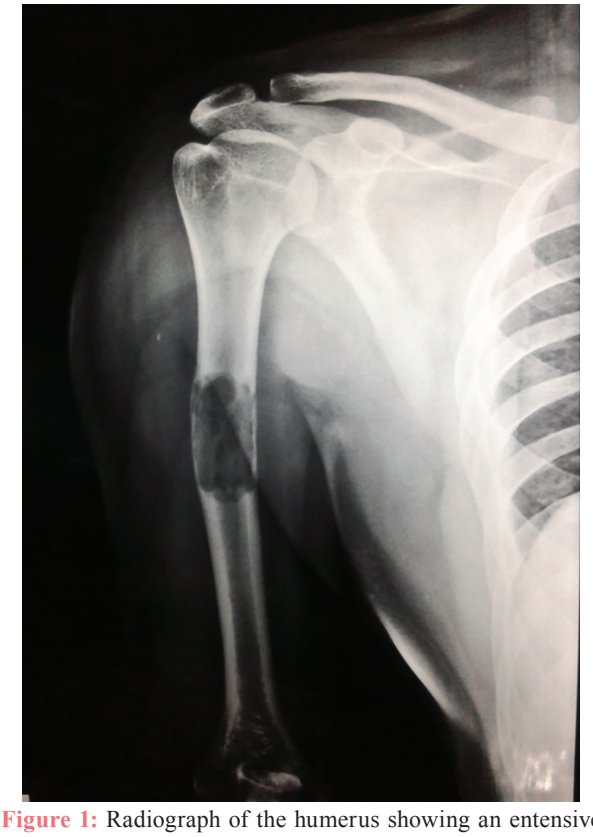

Figure 1: Radiograph of the humerus showing an entensive osteolytic lesion of the right humerus

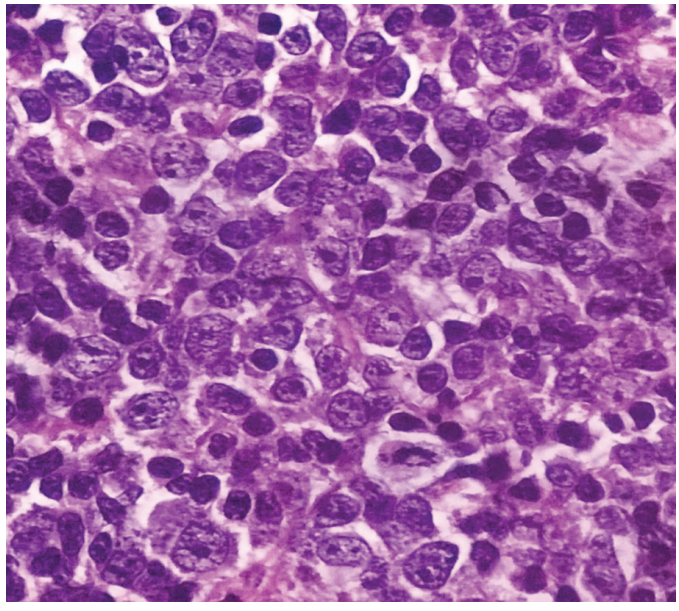

Figure 2: H&E x100 – Section showing sheets of mature plasma cells and few immature forms

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