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ORIGINAL ARTICLE

A Prospective Crossover Triple-Blind Controlled Trial on the Safety and Efficacy of Iranian Recombinant FVIII (Safacto®) Versus Plasma Derived FVIII; A Pilot Study

Eshghi P[1], Abolghasemi H[1], Malek F*[1], Naderi M[3], Panahi Y[2], Habibpanah B[4], Fatohlahzadeh E[4], Gorji F[1]

1. Pediatric Congenital Hematologic Disorders Research Center, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Zahedan University of Medical Sciences, Zahedan, Iran
4. Comprehensive Care Center for Children with Hemophilia, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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*Corresponding author: Malek F,
Email: fmalek7721@gmail.com

ABSTRACT

Background: Considering the increasing number of patients with hemophilia and infrastructure requirements for a comprehensive approach, development of a recombinant factor has become a milestone.
The objective of this study was to assess the safety, efficacy and non inferiority of Safacto (Recombinant factor VIII) compared with plasma-derived factor in the treatment of hemophilia A.
Methods: 10 patients with severe hemophilia A were enrolled in this study. Each patient was treated by a 40-50 IU/kg infusion of either plasma derived or recombinant factor VIII after initiation of each of 4 consecutive hemarthrosis episodes in a triple-blind prospective crossover permuted block randomizing method. Clinical efficacy scale score and in vivo recovery of factor VIII was assessed in each of the treated bleeding episodes. Any adverse event was also recorded.
Results: The mean±SD level of factor VIII in the plasma versus recombinant groups was 111.5±39 and 115±39, respectively without any significant difference. Response scaling method which assessed pain and range of motion revealed equalized scores along with in vivo recovery, hence treatment success rate was comparable in both groups. One non-recurring, mild skin rash reaction occurred simultaneous with the administration of plasma derived factor.
Conclusion: Safacto (r-FVIII) is safe and effective and non-inferior to plasma derived factor VIII in the treatment of hemophilia A related bleeding events.

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Introduction

Hemophilia A is an X-linked bleeding disorder that results from insufficiency of factor VIII (FVIII) coagulant activity [1-4]. Patients with severe disease (FVIII levels <1%) may present with spontaneous bleeding and lack of appropriate treatment leads to life threatening bleeding [1-5].

Factor replacement is the main strategy in the treatment of hemophilia. Currently, the affordable treatment products include plasma derived factor which is originally isolated from pooled human plasma, and FVIII concentrates whom are produced via techniques that employ genetic engineering [1-3]. Various trials revealed comparable efficacy and safety of both products. Synthesis of coagulation factors ensure safe therapy for patients with hemophilia and protect them against blood borne infections [3-5].

In spite of challenges regarding the immunogenicity of recombinant FVIII and great progress in production and viral inactivation methods, the safety of recombinant coagulation factor is much higher compared with plasma derived counterparts [5-9]. The available products are categorized into four generations of recombinant FVIII that are shown in table 1.

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Table 1: Available products categorized into four generations of recombinant FVIII

	1st Generation	2nd Generation	3th Generation	4th Generation
Recombinant technology	+	+	+	+
Chemical virus inactivation		+	+	+
Final product Free from human or animal protein		+	+	+
Cell culture Free from human or animal protein			+	+
Purification with a synthetic ligand				+
Nanofiltration				+

Parallel with the mentioned classifications few products were manufactured with deletion of the B domain which caused reduced immunogenicity [9-17]. The CANAL trial revealed that FVIII products have a similar propensity to produce FVIII inhibitors as plasma derived [9,10].

Management of haemophilia is a major challenge, especially in developing countries, from various aspects such as shortage of product, the cost, and problems with health-related infrastructures [4,11]. Iran as a middle eastern country with about 2767 US dollars per capita gross national product has higher than expected factor consumption index which is more than 2 unit/capita based on the 2011 annual global survey of the World Federation of Hemophilia (WFH) [5]. Since 2014, the treatment strategy of hemophilia in Iran is shifting toward prophylaxis which will impress factor demand. In order to solve the aforementioned issues and exert comprehensive care, Iranian scientists have manufactured SafactoÒ, B domain deleted, albumin free FVIII product in which the cell line culture was Chinese hamester's ovary (CHO) purified with a synthetic ligand whose pharmacological characteristics were registered in the food and drug organization of Iran in 2012 [14-18]. We hypothesize that manufacturing Iranian recombinant factor will have a profound impact on reducing governmental expenditure considering that 99% of hemophilia care disbursement in Iran has been factor supply expenditure [4]. We aimed to compare the efficacy, safety, and non inferiority of recombinant factor versus plasma derived factor.

Patients and Methods

This triple-blind prospective crossover randomized permuted blocking pilot study was conducted in two comprehensive care centers in Iran (Mofid children's Hospital, Tehran, and Ali Asghar Hospital, Zahedan). Investigators and analysts were unaware of the treatment arms in order to prevent any bias in this study. Potential subjects who had the inclusion criteria were identified from the Haemophilia Reference Centre database and were invited to participate. This study was approved by the ethical review committee of shahid Beheshti Medical University and the health ministry in agreement with declaration of Helsinki and Good Clinical Practice. Parent(s)/patient(s), of all study subjects gave informed written consent at the time of study entry. The trial was registered on the Iranian Registry of Clinical Trials (IRCT) web site with registration number 2014082018870N1.

Previously treated patients aged more than one year with severe hemophilia A with a history of experiencing more than 50 exposure days whose clinical biochemistry results were within the normal range were eligible for enrolment.

The exclusion criteria were history of inhibitors (neutralizing antibodies), hypersensitivity associated with any FVIII concentrate or intravenous immunoglobulin, or being affected by another bleeding disorder.

In order to assign allocation concealment appropriately, the corresponding manager of the trial firstly assigned identification numbers to participants then encoded them confidentially. The batches were encoded to A and B, concealed in an opaque sealed envelope not to be revealed until study termination. Randomization was done suing the random permuted block design with blocks with a length of four.

After enrollment, all of the eligible patients were assigned to receive on demand infusion, at dose of 40-50 mg/kg factor VIII for 4 consecutive times randomly (2 times for each kind of product either plasma derived or recombinant).

Each infusion of FVIII was monitored for significant changes in vital signs, other adverse signs or symptoms, and clinical response to treatment. The dose of factor administration was rounded to cost benefit vial, and estimated dosage was fixed for every patient.

Simultaneous with the first FVIII administration, plasma FVIII levels were drawn pre-infusion, and 15 minutes post-infusion and measured in the participating reference laboratory of the Iranian Blood Transfusion Organization.

Efficacy of recombinant factor in our trial was evaluated firstly by hemostatic recovery and by the quantitative self-assessment scaling method, which will be described in detail.

Following the infusion, the actual to expected FVIII recovery ratio at 15 minutes post infusion was calculated, based on the expectation that 1 IU FVIII U/kg body weight would raise plasma FVIII activity by 2%. Hence, a ratio of actual to predicted FVIII recovery>0.66 was considered within the normal limits [19]. Hematological parameters and inhibitor screening were assayed before and one month after termination of study.

Patients were instructed to stay in hospital for 3 hours and could be discharged if symptom free, and were informed of possible adverse reactions.

A clinical efficacy scaling system was designed based upon the patient's self-assessment of pain relief and improved joint mobility. The patients were instructed to give a score of 0 to 2 after assessing the mentioned items every 3 hours for 24 hours. Treatment efficacy was considered as reaching a minimum score of three

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in the first three hours and no need for any further factor infusions thereafter due to pain relief in first eight hours.

Citrated plasma and serum were stored at -70°C and shipped in batches from the participating treatment centers to the reference laboratory. FVIII activity was measured at IBTO center by a one-stage, activated partial thromboplastin time based assay using substrate plasma deficient in FVIII. Citrated plasma was assayed for the presence of inhibitors using a modification of the Bethesda and Nijmegen method. Inhibitor titers were quantitated in the reference laboratory [20].

Data were analyzed using SPSS software, version 18. Quantitative data were expressed as mean, median and standard deviation. T, Mann-Whitney, Roc curve and Pearson's tests were used as appropriated.

Results

Ten patients with mean age of 5.5 year (range: 4-43years) were enrolled. At the end of the trial the drug batches were decoded: A was (Pd FVIII) and B was recombinant (rFVIII).

The mean±SD plasma level of FVIII activity was 111.5±39 U/dl in plasma derived FVIII and 115±39 U/dl in the recombinant FVIII (P=0.753). The mean±SD of actual to predicted recovery for plasma derived FVIII was 1.8±0.35 (0.54-1.75) and 1.1±0.31 for recombinant FVIII (0.66-1.75) (P=0.583). In spite of the mentioned results we used receiver operating characteristic ROC curve in order to determine a cut-off value for our clinical test. The area under the ROC curve, as an important measure of the accuracy of the clinical test was 0.475. The patients' efficacy scaling score of product A and B were comparable with each other: the pain relief score was 10.4±1.8 (4-12) for plasma derived FVIII, 11±1 (7-12) for recombinant FVIII (P=0.142). Joint mobility scores were 10.5 ±2 (4-12) and 11±1 (9-12) for plasma derived FVIII and recombinant FVIII, respectively (P=0.820).

One nonrecurring acute adverse reaction post infusion was detected after plasma derived administration which was manifested by paresthesia.

Equalized scores achieved in coordination with in vivo results revealed comparable efficacy and safety of recombinant factor versus plasma derived FVIII.

Discussion

Unquestionably the principal benefit of r-FVIII for subjects with hemophilia A is access to a source of clotting factor that is not dependent on the availability of human plasma which could be a potential transmissible of blood borne diseases [1-17]. Aforementioned biogeneric recombinant (Safacto®) and plasma derived products' safety and efficacy are completely comparable which is fully correlated with previous studies between the two products.

To the best our knowledge, this study is the first triple blinded prospective crossover clinical trial pilot study, with strongest study design according to the consolidated statement of reporting trials (CONSORT) [18-21].

In addition, most of the similar trials' methodologies are open label clinical trials, in which the researcher knows the full details of the treatment and so does the patient, hence potentially are more vulnerable to bias [18-24].

Evaluation of efficacy is one of our endpoints composed of components such as cessation of bleeding, pain relief, improvement of joint mobility and eventually hemostatic recovery. Clinical efficacy assessment score was based on pain relief and joint mobility, which revealed comparable results in both products. Actual to predicted FVIII recovery which was used as a laboratory efficacy index in our study which was desirable in almost all patients. The ratio of actual to predicted rFVIII recovery was less than 0.66 on 3 occasions.

Bray et al. conducted an open label trial on efficacy and safety of recombinant factor FVIII in untreated patients. Efficacy was evaluated by pharmacokinetics. Mean±SD of recovery to expected ratio was 1.0±0.4, comparable with our results which was 1.0±0.3. Two acute adverse reaction events were occurred. Efficacy and safety of recombinant FVIII was acceptable in their study [19]. Blanchette et al. designed a multicenter, open-label, prospective cohort study to assess pharmacokinetics, efficacy and safety of a plasma-free recombinant FVIII concentrate (ADVATE Ò) in 53 children less than 6 years of age with 50 days of prior FVIII exposure. 90% of the episodes were managed with one or two infusions and their response was rated excellent/good in 93.8% of episodes with a qualitative scoring method for a period of eight hours. Our study and the response scoring method was quantitative with extended observation time up to 24 hours [18].

In another long-term, multicenter study performed by Lusher et al. the safety, efficacy and rate of inhibitor formation of rFVIII B domain deleted (KogenateÒ) treating (PUPs) as a sole therapy was assessed. They used recovery and the subjective assessments using a five-point scale [23].

Apparently structural similarities such as prolonged assessment time and measuring recovery ratio between the mentioned study and ours is evident emphasizing on the importance of quantitative subjective scales.

Although it is difficult to overlook the small number of participants in this pilot study, the rigorous study design and the appropriate data analysis could compensate. On the other hand, since we studied previously treated patients, we were unable to evaluate the product's immunogenicity; the most challenging aspect of hemophilia treatment as this was the main reason for designing the Survey of Inhibitors in Plasma-Product Exposed Toddlers (SIPPET) currently under way. Due to small scale of this pilot study and lack of the power to recommend changes in clinical practice larger multicentre studies before launching the product to the market would be necessary.

Declaration of Conflicting Interests

This research was conducted upon the request of Food and Drug organization of ministry of Health and Medical Education of Iran. The research was financially supported by SAMEN Darou pharmaceutical company which were involved neither in the collection, interpretation, and analysis of the data nor in decision to write and submit of the report.

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ORIGINAL ARTICLE

Is There any Association between Thyroid Autoimmunity and Breast Cancer?

Valizadeh N*[1], Delpak A[2], Eishi Oskuie A[3], Valizadeh N[4]

1. Assistant Professor of Endocrinology and Metabolism, Maternal and Childhood Obesity Research Center, Urmia University of Medical Sciences, Urmia, Iran
2. General Physician, Emam-Khomeini Hospital, Urmia University of Medical Sciences, Urmia, Iran
3. Associate Professor of Hematology/Medical Oncology, Emam-Khomeini Hospital, Urmia University of Medical Sciences, Urmia, Iran
4. Assistant Professor of Hematology/Medical Oncology, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran

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**Corresponding author:* Neda Valizadeh
4th floor, Ershad Street, Emam Khomeini hospital, Urmia University of Medical Sciences, Zip Code: 81351-57157, Urmia, Iran
Tel: +98 443 3459538
Fax: +98 443 3469935
Email: nvalizadeh2008@gmail.com

ABSTRACT

Background: The relationship between thyroid autoimmunity and breast cancer is a challenging subject. We aimed to investigate this association in women with breast cancer.
Methods: In this descriptive study, 41 women with newly diagnosed breast cancer before receiving any pharmacologic treatment and 38 healthy age-matched women were enrolled. Anti TPO Ab(anti-thyroid peroxidase antibodies), FT4(free thyroxine), T3(triiodothyronine) and TSH (thyroid-stimulating hormone) were measured in both groups.
Results: The mean±SD ages in patients with breast cancer and the control group were 41.71±1.73 and 40.03±1.74 years, respectively (P=0.496). There was no statistically significant difference between the mean values of FT4 and T3 in patients with breast cancer (P=0.447) and the control group (P=0.534). The mean TSH level in patients with breast cancer was 4.9±1.7 µIU/ml which was significantly higher than healthy women (1.79±0.15 µIU/ml, P=0.004). The frequency rate of the increased Anti TPO Ab levels (higher than 35 IU/ml) in women with breast cancer was 22% which was significantly higher than the control group (%0, P=0.002), while no statistically significant difference was found between the mean Anti TPO Ab levels between the two groups (61.07±29.73 versus 9.78±0.78, P=0.21). Four cases of subclinical and/or overt hypothyroidism were found in women with breast cancer.
Conclusion: Based on our findings breast cancer patients have higher rates of thyroid autoimmunity. Measurement of FT4 and T3 in all women with breast cancer is not recommended but measurement of TSH and Anti TPO Ab levels seem reasonable.

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Introduction

Breast cancer (BC) is one of the most prevalent malignancies worldwide and also Iran.[1,2] A variety of hereditary, environmental, reproductive and hormonal factors have been proposed in the pathogenesis of BC; although in most of cases the exact etiologic factor has not been well understood.[1,3,4] The relationship between thyroid autoimmunity and BC is a challenging subject. The findings of some studies were in favor of this association but others did not support this idea.[5-8] We designed this study in order to investigate the association between thyroid autoimmunity and BC.

Patients and Methods

In this descriptive study, 41 women aged 20-70 years with newly diagnosed BC who had been referred to the Oncology Clinics of Urmia city or those who had been admitted to Emam Khomeini Hospital of Urmia and had not taken any chemo-radiation therapy previously were enrolled. 38 age-matched healthy women who did

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not have any history of thyroid or breast diseases were selected as the control group. Subjects with history of nephrotic syndrome, liver or renal failure, hysterectomy, oophorectomy, thyroidectomy or previously known thyroid diseases and those who had received levothyroxine, anti-thyroid drugs, Tamoxifen and other hormonal drugs were excluded.

The study was approved by the Ethic Committee of Urmia University of Medical Sciences. In all subjects after obtaining written informed consent, venous blood samples were taken and free thyroxine(FT4), T3(triiodothyronine) and TSH (thyroid-stimulating hormone) were analyzed by ELISA(enzyme-linked immunosorbent assay) method (Pishtazteb kite, Tehran, Iran). Anti TPO Ab was measured using ELISA method (Monoind kite, USA). The normal range of Anti TPO Ab was less than 35 IU/ml and levels higher than 35 IU/ml were in favor of thyroid autoimmunity. The reference ranges of FT4, T3 and TSH hormones were 0.7-1.8 ng/dl, 0.8-2.1 ng/ml and 0.3-5.5 µIU/ml respectively. Postmenopausal women had not menstruated for at least six months prior to the study.

The patients' information including demographic findings, menopausal status and laboratory tests results were recorded and data were analyzed using SPSS software, version 16. Also, *t*, Mann-Whitny U, Fisher, and Pearson's Chi-square tests were used as appropriated. P<0.05 was considered as significant.

Results

The mean±SD aged of patients with BC and the control group were 41.71±1.73 and 40.03±1.74 years, respectively (P=0.496). As shown in Table 1, 36.8% of patients with BC and 41.46% of healthy women were in the postmenopausal state (P=0.674). There were no significant differences between the mean values of FT4 and T3 in patients with BC (P=0.447) and the control group (P=0.534). The mean±SD TSH level in patients with breast cancer was significantly higher than healthy women (4.9±1.7 µIU/ml *vs.* 1.79±0.15 µIU/ml, P=0.004).

The percentage of women with abnormal TSH levels including higher than 5.5 µIU/ml and lower than 0.3 were 9.8% and 2.4% respectively in patients with BC, whereas no woman with abnormal TSH level was found in the control group (P=0.084).

The frequency rate of the increased Anti TPO Ab levels (higher than 35 IU/ml) in women with breast cancer was 22% which was significantly higher than healthy women (%0, P=0.002) but no statistically significant difference was found between the mean values of Anti TPO Ab between patients with BC and the control group (61.07±29.73 versus 9.78±0.78, P=0.21).

Table 2 shows the percentage of subjects with normal, increased and decreased concentrations of FT4 and TSH in patients with BC (P=0.168) and healthy women (P=0.084). Among the four patients with BC with high TSH levels, two cases of subclinical hypothyroidism (increased TSH and normal FT4 levels) and two cases of overt hypothyroidism (increased TSH and low FT4 levels) were detected. All patients in both groups had normal T3 levels.

Discussion

In this study, although the mean values of thyroid hormones (FT4 and T3) and Anti-TPO Abs did not differ in women with BC and healthy controls, the prevalence of elevated Anti-TPO Ab (more than 35 IU/ml) was significantly higher in women with BC. Women with BC had higher TSH titers than healthy women. We also found four patients with hypothyroidism (subclinical and/

Table 1: Demographic characteristics and thyroid function tests of women with breast cancer and healthy women

Variable	Patients with breast cancer (n=41)	Healthy women (n=38)	P value
Age (years)	41.71±1.73	40.03±1.74	0.496
Menopause (%)	14(36.8%)	17(41.46%)	0.674
TSH (µIU/ml)	4.9±1.7	1.79±0.15	0.004
FT4 (ng/dl)	1.23±0.04	1.27±0.03	0.447
T3 (ng/ml)	1.16±0.04	1.13±0.04	0.534
Anti TPO Ab (IU/ml)	61.07±29.73	9.78±0.78	0.21
Anti TPO>35 IU/ml	9(22%)	0(0%)	0.002

Data are expressed as mean ± SE (Standard error), FT4 (free thyroxine), T3 (triiodothyronine), TSH (thyroid-stimulating hormone), Anti TPO Ab (anti-thyroid peroxidase antibodies)

Table 2: Comparing thyroid function tests between women with breast cancer and healthy women

Variable		Breast cancer (n=41)	Control group (n=38)	P value
TSH	Increased	4(9.8%)	0(0%)	0.084
	Decreased	1(2.4%)	0(0%)	
	Normal	36(87.8%)	38(100%)	
FT4	Increased	0(0%)	0(0%)	0.168
	Decreased	2(4.9%)	0(0%)	
	Normal	39(95.1%)	38(100%)	

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or overt) in patients with BC.

While limited studies did not find any association between thyroid autoimmunity and BC[6,9,10] growing evidence has demonstrated this relationship.[11-16]In a study by Jiskra and co-workers, the prevalence of increased levels of Anti-TPO Abs and anti thyroglobin antibodies (Anti Tg Abs) were significantly higher in women with breast cancer compared to those women with colorectal malignancies and women without cancer.[14]

In a prospective study by Giani and colleagues, the researchers found that the prevalence of thyroid diseases especially Hashimoto thyroiditis and nontoxic goiter was significantly higher in women with BC compared with healthy women. However they did not find any significant difference between mean values of FT4, FT3 and TSH in women with BC and the control group.[11]

In another study by Turken and colleagues, although no significant difference was seen in mean FT4, FT3, TSH levels and Anti Tg Abs between women with BC and healthy controls, the mean Anti-TPO Abs levels was significantly higher in patients with BC. Higher frequency of autoimmune thyroiditis and goiter was also found in patients with BC.[8]

Gogas and colleagues also found higher incidence of thyroid autoimmunity in patients with BC.[13]

Giustarini et al observed significant relationship between BC and thyroid autoantibodies and they recommended evaluation of autoimmune thyroid diseases in patients with nodular breast disease before surgery for earlier diagnosis and treatment of hypothyroid state.[12]

Studies by Rasmusson[15] and Shering[16] and co-workers have also supported the hypothesis of relationship between thyroid autoimmunity and breast cancer.

In some studies BC patients with increased TPO Abs had favorable outcome.[17] Although the precise mechanism by which thyroid autoimmunity plays a role in BC pathogenesis is unknown, but similar abilities of thyroid follicles and breast epithelial cells for concentration of iodine via natriume iodide symporter expression, existence of TSH receptors in the breast tissue, interaction of thyroid autoantibodies with receptors of breast neoplasms and thyroid releasing of some hormonal and growth factors that affect mammary glands are some possible explanations.[11,13,14,18]

Jiskra and colleagues suggested that increased iodine intake in breast tissue stimulates thyroid autoantibodies and has a favorable influence on patients survivals and response to treatment. In their study TSH levels greater than 3.5 mIU/l were associated with lower carcinoembryonic antigen levels in patients with BC.[14]

One of the limitations of this study was the small sample size and we cannot definitely support the association between thyroid dysfunction and breast cancer.

Conclusion

Based on our result, thyroid autoimmunity may be associated with breast cancer and women with BC have higher rates of positive Anti TPO Abs compared to healthy women. We do not recommend routine measurement of FT4 and T3 in BC patients, but measurement of TSH and Anti TPO Ab levels seems reasonable in these individuals for earlier discovering of autoimmune thyroid dysfunction. We also suggest further research to investigate the role of thyroid autoantibodies in predicting breast cancer outcome.

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Conflict of Interest: None declared.

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ORIGINAL ARTICLE

The Influence of Fetal Hemoglobin on Clinical and Hematological Variables of Children and Adolescents with Sickle Cell Anemia in Basra, Southern Iraq

Badr AK*[1], Hassan MK[2]

1. Pediatrician, Thi-Qar Health Directorate
2. Professor, Department of Pediatrics, Basra Medical College, Basra, Iraq

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*Corresponding author: Meaad K. Hassan
Basra Medical College, Basra, Iraq
Tel: +96 47801000174
Email: alasfoor_mk@yahoo.com

ABSTRACT

Background: There are many parameters that modulate the severity of sickle cell anemia. Fetal hemoglobin (Hb F) is one of these major variables. However, its effect is clinically inconsistent. We conducted a descriptive study to assess the influence of Hb F on clinical events and hematological variables in patients with sickle cell anemia.
Methods: 151 patients with sickle cell anemia with a stable condition, aged 1-18 years, were recruited from March through November 2010. The results of complete blood count and Hb F level and various clinical variables were recorded.
Results: Of the 151 patients, the Hb F was more than 20%, 10-20%, and less than 10% in 77 (51%), 60 (39.7%), and 14 (9.3%) patients. A significant negative association was reported between Hb F level and frequency of painful crisis (95% CI=0.05-0.96, OR=0.22), acute chest syndrome (95% CI=0.01-0.43, OR=0.07) and frequency of hospitalizations (95% CI=0.03-0.85, OR=0.11). There was a significant positive association between hemoglobin level (95% CI=2.14-27.17, OR=7.63) and splenomegaly (95% CI=1.37-57.4, OR=12.88) with Hb F level.
Conclusion: In children and adolescents with sickle cell anemia, the higher the Hb F levels, the lesser clinical complications of the disease would be. Therefore, patients with low Hb F need close follow-up and monitoring since early age to detect complications as early as possible and consider use of disease modifying agents.

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Introduction

Hemoglobinopathies, mainly sickle cell disease (SCD), are challenging health problems in the population of Basra, Southern Iraq where around 6.48% of the population are carriers of sickle cell gene with a gene frequency of 0.0324%.[1] Individuals with sickle cell anemia (SCA) are characteristically asymptomatic until the second half of the first year of life. This lack of clinical expression of the hemoglobin (Hb) SS genotype during early postnatal life can be explained by production of a sufficient quantity of fetal hemoglobin (Hb F) that limits clinically important sickling process.[2]

Properties of Hb F which help in attenuating the severity of SCA are due to the lack of participation of Hb F molecules in polymerization of Hb S. As a result higher amount of Hb F in a cell causes a lower Hb S concentration.[3] The preventive effects of Hb F on Hb S polymerization appear to be concentration dependent.[4] The effect of Hb F on Hb S may affect other red blood cells' characteristics directly or indirectly as Hb F level affects the RBC adhesive properties of patients with SCA.[5] High levels of Hb F may reflect increased synthesis resulting in mild disease, or greater amplification through accelerated destruction of cells containing no Hb F, a manifestation of more severe disease.[2] Levels of Hb F influence the age at which symptoms develop and partially determine the risk of acute splenic sequestration crises (ASSC), stroke, acute chest syndrome (ACS), leg ulcers,

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pain crises, loss of spleen function, and mortality.[2,6,7]

It was suggested that some complications of SCD such as vaso-occlusion and blood viscosity are strongly associated with Hb F level; whereas, complications related to the degree of hemolysis are dependent on Hb F to a lesser degree.[8]

Although many studies have considered Hb F level ≥10% as high Hb-F,[9-11] other studies have defined levels of more than 5.4% as high.[12] The impact of Hb F on clinical course of the disease is variable and not consistent as Hb F percentage reaching 20% may be found in patients with severe disease.[13]

We aimed to study the level of Hb F among children and adolescents with SCA and the association between Hb F level and clinical variables and hematological parameters reported in these patients.

Patients and Methods

This descriptive study was done on children and adolescents with SCA (homozygous Hb S) over a 9-month period (from the first of March until the end of November 2010). 151 children and adolescents with SCA registered at the Center for Hereditary Blood Diseases, which is the only center caring for these patients in Basra were the study was done. Patients were in a stable condition with an age range of 1-18 years, consisting of 84 boys and 67 girls. Patients with sickle cell/ β-thalassemia and patients on Hydroxyurea (HU) were excluded from the study.

Baseline steady state was defined as a steady hemoglobin and hematocrit level reported during 2-3 clinical visits with an interval of 4-6 weeks and a condition of wellbeing with no symptom or sign suggestive of crisis, infection, and other diseases confirmed by thorough history and physical examination.[14,15] All Information including socio-demographic and clinical data were taken from patients and/or their parents or other caregivers in addition to patients' records.

Physical examination was performed for all patients. Body weight and height were measured and body mass index (BMI) was calculated for all patients. According to BMI Classification of Children and Adolescents; the patients were considered underweight when BMI was <5th percentile, normal weight with a BMI between 5th-84th percentile, at risk for overweight with a BMI of 85th-94th percentile, and overweight when BMI was ≥95th percentile.[16] Moreover, follow-up of these patients and their complications were recorded during the study period. An informed consent was obtained from one of the parents/caregivers before enrollment in the study. The study was approved by the Research Ethics Committee of Basra Medical College.

Disease severity was assessed so that severe disease was defined as patients requiring hospitalizations for SCD-related complications ≥3 times/year, acute painful crises requiring hospitalization of ≥3 times/year, frequent blood transfusion ≥3 times/year, history of ACS, ASSC or avascular bone necrosis.[2,14]

The diagnosis of SCA was confirmed by High Performance Liquid Chromatography (HPLC), (VARIANT™, β- Short Programs; Bio-Rad Laboratories, Hercules, CA, USA). The relationship between Hb F level and clinical events was assessed by dividing patients into three groups based on Hb F level; <10%, 10-20% and >20%.

Estimation of hemoglobin, white blood cell (total and differential), platelets counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were done by an Automated Hematology Analyzer CBC+3 part diff. Symex KX-21N.

Other investigations including echocardiography, electrocardiography, chest x-ray, urinalysis, urine culture and ultrasonography were done when indicated.

Statistical analysis were carried out using the Statistical Packages for Social Sciences (SPSS Inc., Chicago, IL, USA) software version 17.0. Data were expressed as mean±standard deviation (SD). Comparisons of proportions were performed by crosstab using Chi-square test. Comparisons between groups were assessed using one-way analysis of variance (ANOVA). Logistic regression analysis was also done for the analysis of different variables, the 95% confidence interval (CI) were assessed. For all quantitative variables P<0.05 was considered as statistically significant.

Results

In this study, 151 children and adolescents with SCA were evaluated with a mean±SD age of 7.6±4.94 years for boys and 8.4±4.04 years for girls. The mean±SD Hb-F level was 19.65±7.42 with no significant difference between boys (19.97±7.50) and girls (19.26±7.30) (P>0.05). The mean±SD Hb S was 70.76±7.39. The Hb F level was more than 20%, 10-20%, and less than 10% in 77 (51%), 60 (39.7%), and 14 (9.3%) patients.

Pallor, jaundice, acute painful episodes, ACS, and history of hospitalizations were significantly higher among patients with Hb F <10% (P<0.05). While splenomegaly was reported in a significantly higher number of patients with Hb F more than 20% (table 1). Persistent splenomegaly was found in 30.10% of patients beyond 6 years of age. Aplastic crisis, ASSC, priapism and stroke were not reported among patients enrolled in this study.

The mean frequency of painful episodes, total blood transfusions, frequency of hospitalizations/year and total hospitalizations were significantly higher among patients with Hb F less than 10% (P<0.01). There was no significant difference between age of presentation and number of blood transfusions/year among patients with different Hb F levels (P>0.05, table 2).

Concerning hematological parameters, the mean±SD Hb level for the studied patients was 8.9±6.83 g/dL and it was significantly higher among patients with Hb F levels more than 20% (P<0.01). Other parameters were not statistically significant among patients with different Hb F levels (P>0.05, table 3).

History of blood transfusion was positive in 62 (41%) patients. Blood transfusions were significantly more frequent among patients with Hb F <10% (P<0.05).

After subjecting various variables to regression analysis, there was a significant positive association between Hb

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Table 1: Distribution of SCA patients according to the level of fetal hemoglobin and clinical events

Clinical events	Hb F level			Total No %	P value
	<10% (No. 14) %	10-20% (No. 60) %	>20% (No. 77) %		
Pallor	10 (71.4)	27 (45)	26 (33.8)	63 41.72	0.005
Acute painful episodes	14 (100)	56 (93.3)	63 (81.9)	133 88.07	0.001
Jaundice	6 (42.9)	12 (20)	11 (14.3)	29 19.20	0.000
ACS	6 (42.9)	13 (21.7)	6 (7.8)	25 16.55	0.007
Infections	5 (35.7)	13 (21.7)	21 (27.3)	39 25.82	0.121
Heart failure	1 (7.1)	4 (6.7)	4 (5.2)	9 5.96	0.981
Splenomegaly	2 (14.3)	16 (26.7)	33 (42.9)	51 33.77	0.001
Hepatomegaly	1 (7.1)	7 (11.7)	7 (9.1)	15 9.93	0.721
Gall stone	1 (7.1)	7 (11.7)	2 (2.6)	10 6.62	0.096
Splenectomy	0 (0.0)	2 (3.3)	0 (0.0)	2 1.32	
Bone necrosis	0 (0.0)	2 (3.3)	0 (0.0)	2 1.32	
History of hospitalization	12 (85.7)	39 (65)	40 (51.9)	91 60.26	0.021
BMI (<5th percentile)	4 (28.57)	21 (35)	21 (27.27)	46 (30.46)	0.942

Table 2: Selected clinical variables in relation to fetal hemoglobin level

Variables*	Hb-F level			Total (151)	P value
	<10% (No. 14)	10-20% (No. 60)	>20% (No. 77)		
Age at first presentation(years)	3.40±1.65	3.91±2.05	3.60±1.76	3.73±1.87	0.138
Acute painful episodes /year	8.52±5.72	7.64±6.2	4.80±4.3	6.31±5.45	0.005
BT/year	1.85±2.68	1.13±1.81	0.94±1.91	1.10±1.96	0.314
Total BT	20.71±24.54	7.03±9.49	5.29±11.47	7.41±13.16	0.001
Hospitalization/year	3.15±4.92	0.69±1.07	0.31±0.41	0.72±1.80	0.029
Total hospitalization	23.10±4.92	5.63±8.47	2.24±3.54	5.52±11.22	0.000

*Variables are presented as mean±SD; ANOVA test was used

Table 3: Distribution of SCA patients according to the level of fetal hemoglobin and hematological variables

Hematological Variables *	Hb-F level			P value
	<10 (N. 14)	10-20 (N. 60)	>20 (N. 77)	
Hb(g/dl)	7.85±1.25	8.17±1.25	8.69±1.40	0.000
PCV	23.64±3.71	24.23±3.75	25.55±4.50	0.120
MCV(fl)	80.42±5.72	78.69±5.62	77.81±5.36	0.180
MCHC(Hb/l RBC)	316±28.45	307±33.28	303±39.31	0.785
Retic(%)	6.52±2.42	6.63±2.38	6.16±2.70	0.653
MCH	26.29±3.22	25.35±3.05	24.61±2.62	0.783
WBC($\times 10^9$)	11.007±3.07	9.778±3.69	9.825±3.38	0.265
Platelets($\times 10^9$)	336.357±126.94	300.083±126.49	279.93±106.90	0.132

*Variables are presented as mean±SD; ANOVA test was used

F level with splenomegaly and total Hb level (table 4). Moreover, a significant negative association was found between pallor, jaundice, acute painful episodes, ACS and history of hospitalization with Hb F levels.

Discussion

Hemoglobinopathies are common hematologic disorders throughout the Arab world due to high level (25-60%) of consanguineous marriages.[17] SCA is the most common hemoglobinopathy in Basra, followed by β-thalassemia.[1]

A high mean Hb F was reported among patients with SCA in Basra, and more than half of them had Hb F level more than 20%. This percent is lower than that reported by Chopra et al. in Eastern Province of Saudi Arabia, where 75% of patients with SCA had Hb F level above 20%.[18] In the United Arab of Emirates, those with Arab-Indian haplotypes had a mean Hb F level of 27% and a milder clinical course compared to those with the African haplotypes, Bantu and Benin (mean Hb-F level of 11.3%) who had a severe clinical presentation.[19] Mpalampa and colleagues in Uganda reported that only 37% of children aged 1-18 years had Hb F levels of more than 10% (mean level 9%).[9]

Frequency of hospitalizations was significantly associated with increased Hb F levels. Mpalampa and co-workers reported a significant negative correlation between Hb F level and the total number of blood transfusions, all cause hospitalizations and severe pain episodes in the past year.[9] In addition, it has been reported that in India patients with SCD with Hb F more than 20% had a significantly lower morbidity mainly in terms of hospitalization and painful episodes.[20] However, Darbari and colleagues in the USA did not report a significant impact of Hb F on vaso-occlusive pain crises.[21]

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Table 4: Logistic regression analysis of selected variables with fetal hemoglobin

Variables	95% CI	OR	P value
Pallor	0.05-0.71	0.20	0.032
Jaundice	0.03-0.92	0.18	0.044
VOC	0.05-0.96	0.22	0.029
ACS	0.01-0.43	0.07	0.049
Infections	0.46-12.7	2.42	0.098
Heart failure	0.08-18.1	3.78	0.125
Splenomegaly	1.37-57.4	12.88	0.004
Hepatomegaly	0.21-25.6	2.24	0.095
Gall stones	0.02-6.56	0.37	0.651
Splenectomy	0.19-2.19	0.75	0.787
Bone necrosis	0.21-2.21	0.51	0.921
History of hospitalizations	0.03-0.85	0.18	0.025
Hb level	2.14-27.17	7.63	0.004

We found a significant association between Hb F level and ACS. Increased levels of Hb F decrease intracellular polymerization of Hb S which increases total Hb and oxygen saturation and decreases chest pain and severity of ACS. This is in agreement with a previous report which showed that high Hb F level decreased the attacks of ACS.[22]

Another finding was the positive association between higher Hb F level and splenomegaly. In Italy, researchers found that splenomegaly was present in 28% of adult patients with SCA due to lower degree of tissue infarction in Italian patients.[23] While others found that splenomegaly was prevalent in 28.9% of patients over 6 years of age with SCA in Lebanon [24] which was comparable with our study (30.1%). Parmar and Likhar in India reported higher Hb F levels among patients with SCD and splenomegaly at different age groups compared with patients with no splenomegaly and concluded that Hb F is one of the important etiological causes of persistence of splenomegaly.[25] This probably suggests that higher Hb F levels may have an ameliorating effect on sickling of RBCs and lower frequency of splenic infarctions.[18,26]

We found that blood transfusions were less frequent as Hb F levels increased, although logistic regression analysis did not reveal such a significant association. Mpalampa and colleagues have reported a significant association between Hb F level and frequency of blood transfusions.[9] Higher Hb F among patients with SCD is a well-known factor to decrease overall hemolysis and hence increased survival of RBCs containing relatively more Hb F is related to degree of polymer contents in RBCs.[27]

Attaining weight and growth was not significantly different among patients with SCA of different age groups in relation with Hb F level. However, another study showed that high Hb F maintains growth with a significant positive association between Hb F level and BMI; as more frequent VOC decreases appetite and retards growth. The researchers postulated that low Hb F increases hemolysis and metabolic requirements for erythropoiesis that increases the risk of poor growth.[28]

Although Hb F modulates the phenotype of SCA, Steinberg and colleagues have reported that the concentration of Hb F within the individual RBC is more important than the level of Hb F in the blood. Moreover, distribution Hb F within the cell is another important variable that affects Hb S polymerization and as a result may affect disease severity.[29]

A significant association between Hb F level with total Hb was reported in this study. The sickled RBCs become less deformable, leading to increased hemolytic breakdown particularly in the small arterial capillary bed, which results in local increase in ionized and non-ionized calcium concentration. This forms a greater population of RBCs with reduced flexibility causing a fall in Hb level; whereas, increased Hb F inhibits the aggregation of Hb S that can be clinically significant. However, other studies could not find a significant association between hematological parameters and Hb F level.[30,31]

A few limitations need be addressed for the current study. First, the sample size was relatively small. Secondly haplotype testing was not available which is important in assessing severity of the disease. Despite these limitations, the results of this study are still comparable to published data.

Conclusion

It can be concluded that children and adolescents with SCA in Basra have a high Hb F level that significantly decreases many important clinical complications of the disease, although the influence on hematological variables was less prominent. Therefore, we recommend that children with low Hb F should have closer follow-up and monitoring since early age to detect complications earlier and consider use of disease modifying agents such as hydroxyurea.

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ORIGINAL ARTICLE

Analysis of Expression of SIRT1 Gene in Patients with Chronic Myeloid Leukemia Resistant to Imatinib Mesylate

Ghotaslou A[1], Abbasian S*[1], Ghasemi A[1], Nadali F[2]

1. Hematology Department of Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran
2. Tehran University of Medical Sciences, Tehran, Iran

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**Corresponding author:* Sadegh Abbasian, MD; Hematology Department Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran.
Email: abbasian_sadegh@yahoo.com
Tel: +98 9189482981

ABSTRACT

Background: Chronic myeloid leukemia is a clonal myeloproliferative disease which is characterized by bcr/abl translocation. With the emergence of tyrosine kinase inhibitors such as imatinib mesylate, significant improvement has been made in treatment of this disease. However, drug resistance against this medicine is still an obstacle. SIRT1 is a gene with deacetylase activity which has been detected to have increased expression in many cancers. We aimed to determine if SIRT1 expression could play a role in the emergence of drug resistance in patients with chronic myeloid leukemia being treated with imatinib mesylate.
Methods: 48 patients with chronic myeloid leukemia referred to Dr. Shariati Hospital, Tehran, Iran, were studied. A venous blood sample of patients was collected, RNA was extracted and then cDNA were synthesized. SIRT1 gene expression was done by real-time PCR. The ratio of SIRT1 expression to ABL control gene was calculated. After calculation of CT for target gene and control gene, ΔCT was calculated. The results of SIRT1 expression levels in patients with chronic phase of CML were compared with that of the control group.
Results: 48 patients with chronic myeloid leukemia aged 15-64 years (mean: 40 years) were enrolled. 59% of the participants were men. The highest and lowest mean BCR-ABL expressions in drug-resistant patients were 1% and 57%, respectively. The results of analyzing the value of ΔCT for SIRT1 gene revealed that patients who were drug-resistant to imatinib mesylate had a lower value of ΔCT for SIRT1 than those who were not drug-resistant ($P<0.05$).
Conclusion: SIRT1 gene expression in patients resistant to imatinib mesylate was significantly higher than patients who were not drug-resistant.

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Introduction

Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disease that is characterized by presence of BCR-ABL fusion gene. This oncogene itself results from chromosomal translocation between chromosomes 9 and 22, and the resulting chromosome is known as the Philadelphia chromosome.[1,2]

This disease is a myeloproliferative neoplasm which involves erythroid and myeloid cell lines. The average age at diagnosis is 53 years but all age groups could be affected. CML begins as a chronic disease which eventually may spread to accelerated phases.[3-5]

At present remarkable advances in the treatment of many cancers have been made; however, cases such as drug resistant CML and cancer progression towards advanced stages are still considered as unresolved issues.[6,7] Drug resistance and disease progression are still unsolved concerns in treatment of patients with CML. Introduction of tyrosine kinas inhibitors made a fundamental progress in treatment of CML. Over 70% of patients in the chronic phase of the disease gained complete cytogenetic response; hence, partial remission was acquired after treatment with tyrosine kinase inhibitors.[8] Despite the relative success of tyrosine kinas inhibitors in controlling

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the disease, they failed in eradication of the disease at the level of stem cells. This has led to increased threat of drug resistance and relapse.[9]

Imatinib mesylate is a tyrosine kinase inhibitor that is designed for treatment of malignant CML clones. However, primary and secondary drug resistance to the imatinib reduces the effectiveness of this drug.[10] Mechanisms of drug resistance in CML are unknown. Treatment by this drug is able to create complete cytogenetic remission in the chronic phase of the disease, but not capable of eliciting cytogenetic response in blastic crisis.[11,12]

SIRT1 functions as a deacetylase and its gene is expressed in many malignancies. The activity of the promoter of this gene in CML cells leads to survival and proliferation of these cells. This action is related to multi deacetylase characteristics of SIRT1 gene product.[13] Imatinib mesylate relatively reduces the SIRT1 expression outside the laboratory. The activity of this gene may play a role in keeping CML cells alive for chemical treatment.[14]

In hematopoietic progenitor cells whose BCR-ABL are active, SIRT1 expression can be observed actively as well. Further studies on the expression of this gene may be required to find a therapeutic target for drug resistance in cancers.[15] Excessive increase of SIRT1 product in primary tumors and hematological malignancies has been detected. Inactivation of this gene inhibits the growth of cancer cells and hence increases apoptosis in these cell lines.[16,17]

In the present study, the correlation between SIRT1 gene expression and drug resistance in patients who received imatinib mesylate will be analyzed. Furthermore, the correlation between the patients' response to this medication and pattern of gene expression will be discussed.

Patients and Methods

This study was conducted in the Research Center of Blood and Stem Cell Transplantation of Dr. Shariati Hospital in Tehran, Iran, to investigate the relationship between the expression of SIRT1 gene and drug resistance in patients with CML. 48 individuals were enrolled into the study. Moreover, 10 samples of bone marrow of healthy individuals were used as the control group. All participants in the study gave their written informed consent and the authors did their best to keep the the participants' data confidential.

Patients' RNA was extracted using TRIZOL kit (Sigma, USA). Subsequently, cDNA synthesis was performed using primers of Random Hexamer. SIRT1 gene expression analysis on patients' samples and control group was done using real time-PCR method. The results of SIRT1 expression levels in patients with chronic phase of CML were compared with that of the control group. Data were analyzed by SPSS software, version 18. The data about different phases of the disease and the patients' response to treatment were collected from the patients' medical records.

The result of this technique was reported by calculating the ratio of SIRT1 target gene expression to ABL control gene as a relative quantitative measurement. For Statistical analysis of the data in Light Cycler, the software program called "Second Derivative Maximum Method" was used. The method automatically determines cross point of each sample. In this method, the ABL gene was used as control gene. Since the result should have been relative quantitative, the ratio of SIRT1 expression to ABL control gene was calculated. After calculating CT for target gene and control gene, ΔCT was calculated.

Required primers for SIRT1 and ABL genes were designed. Real-time PCR test was conducted in 45 cycles on control group and the patients by using thermos Light cycler (Roche Diagnostics, Mannheim, Germany) and SYBR® Premix Ex Taq™ (Perfect Real Time) kit. In order to evaluate the changes in gene expression, $2^{(-\Delta\Delta Ct)}$ was calculated after estimation of CT for ABL gene expression as well as SIRT1 gene expression in samples obtained from the patients and the control group. Then, by use of formulas 1-1 to 1-4 and Excel software. $2^{(-\Delta\Delta Ct)}$was calculated for the changes of SIRT1 gene expression in two groups of drug-resistant (or Not Achieved MMR) and drug-sensitive (or Achieved MMR). The criteria for drug-resistance to imatinibmesylate was complete molecular response or (major molecular response) after 18 months of treatment with Imatinib based on the National Comprehensive Cancer network (NCCN) and European Leukemia Net (ELN) guidelines.[18] Furthermore, in order to define the criteria for complete molecular response in patients, the formula of BCR-ABL to ABL expression level was used. If this ratio is lower than 1%, the patients will be considered as appropriate responders. Likewise, this ratio must be re-calculated after 18 months of treatment.[19]

Formulas 1-1 to 1-4 were used to calculate the changes of gene expression, as follows:

Formula 1-1

$$\Delta CT_{\text{target gene in treated sample}} = CT_{\text{target gene in treated sample}} - CT_{\text{foot house in treated sample}}$$

Formula 2-1

$$\Delta CT_{\text{untreated sample}} = CT_{\text{target gene in untreated sample}} - CT_{\text{foot house gene in untreated sample}}$$

Formula 3-1

$$\Delta\Delta CT_{\text{target gene in treated sample}} = \Delta CT_{\text{target gene in treated sample}} - \Delta CT_{\text{target gene in untreated sample}}$$

Formula 4.1

$2^{(-\Delta\Delta CT)}$=The ratio of changes in gene expression in treated sample to untreated sample

Table 1 shows the primer sequence for two genes of SIRT1 and ABL.

Results

We found that all CML patients were in the chronic phase. The patients were all treated with imatinib mesylate. They were categorized into two groups of imatinib-sensitive and imatinib-resistent based on the patterns of treatment responses.

We studied 48 patients diagnosed with CML. The patients aged from 15-46 years, with a mean age of 40 years. 28 (59%) patients were men. The minimum and maximum length of treatment for the participants in the study was 2 and 8 years, respectively. The average BCR/ABL gene expression in patients resistant to imatinib was

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Table 1: Primer sequence for two genes of SIRT1 and ABL

Gene symbol	Sequence (5' to 3')	Primer	Size
ABL-F	TGGAGATAACACTCTAAGCATAACTAAAGG	Forward	Bp124
ABL-R	GATGTAGTTGCTTGGGACCCA	Reverse	
SIRT1	AGGATAGAGCCTCACATGCAA	Forward	Bp104
SIRT1	TCGAGGATCTGTGCCAATCATA	Reverse	

14%, while the minimum and maximum level of BCR/ABL expression in patients was 1 and 57%, respectively. 10 Patients showed complete molecular response to imatinib mesylate and this group was considered as sensitive to treatment. The expression of BCR/ABL in these patients was from less than 1% to undetectable levels.

Comparing the level of SIRT1 gene expression in the two drug-sensitive and drug- resistant groups showed that the amount of ΔCT was lower in the drug-resistant group than the drug- sensitive group. This shows lower expression of SIRT1 in the drug- sensitive group and reflects that SIRT1 expression in the drug-resistant group was significantly higher ($P<0.05$).

Figure 1 shows that in order to ensure the specificity of the real-time PCR products, melting curves were plotted after each working run. In all 48 samples, there was a peak in the melting curves which indicated the specificity of the real-time PCR products. This melting curve diagram shows the performance of SIRT1 and ABL primer. As shown, the curve of all products does show one peak.

In order to draw a standard curve, dilutions of 0.1, 0.01, and 0.001 were prepared from primers of SIRT1 and ABL. After conduction of the test, amplification curves were plotted (figures 2 and 3).

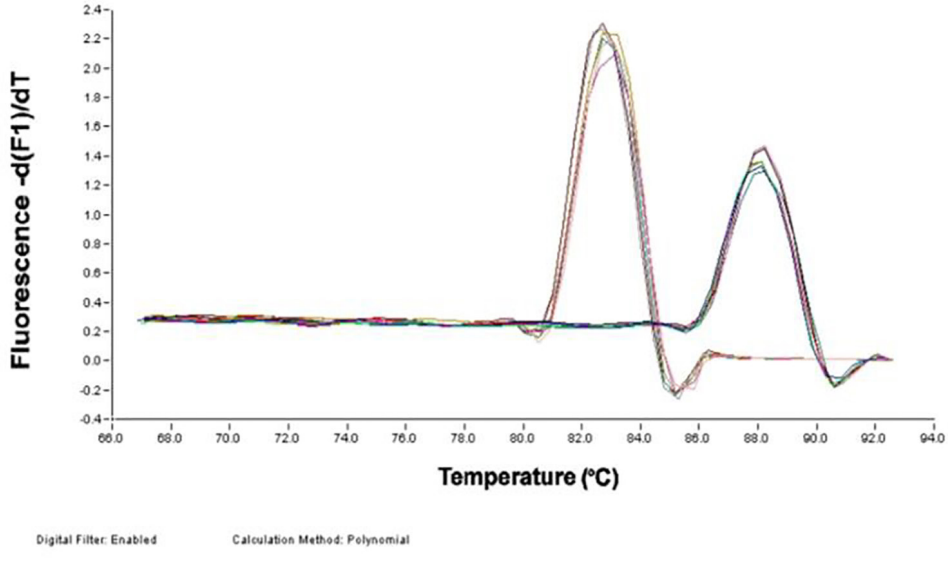

Figure 1: The melting curve of real-time PCR products

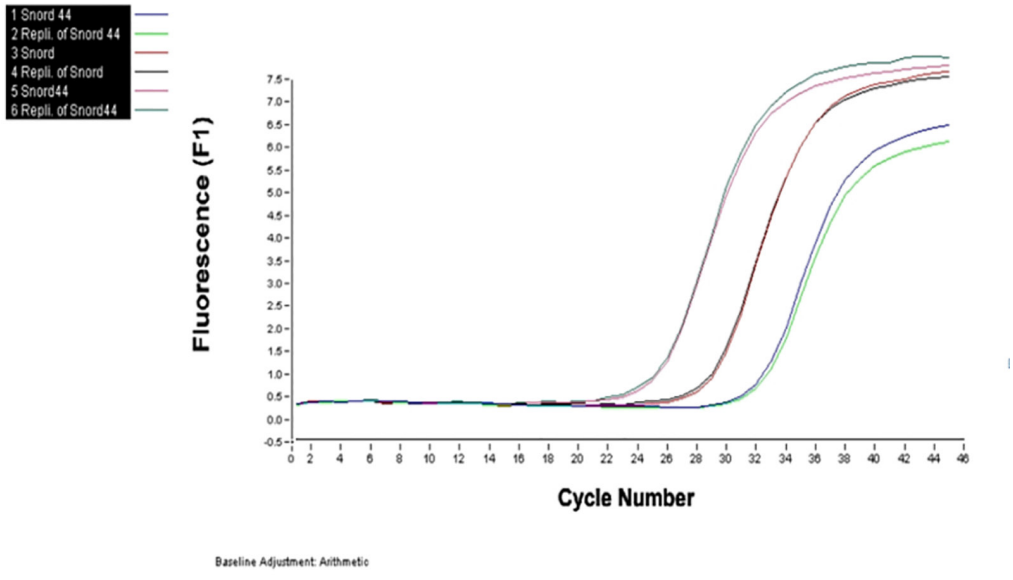

Figure 2: SIRT1 and ABL primers curve used to standard diagram

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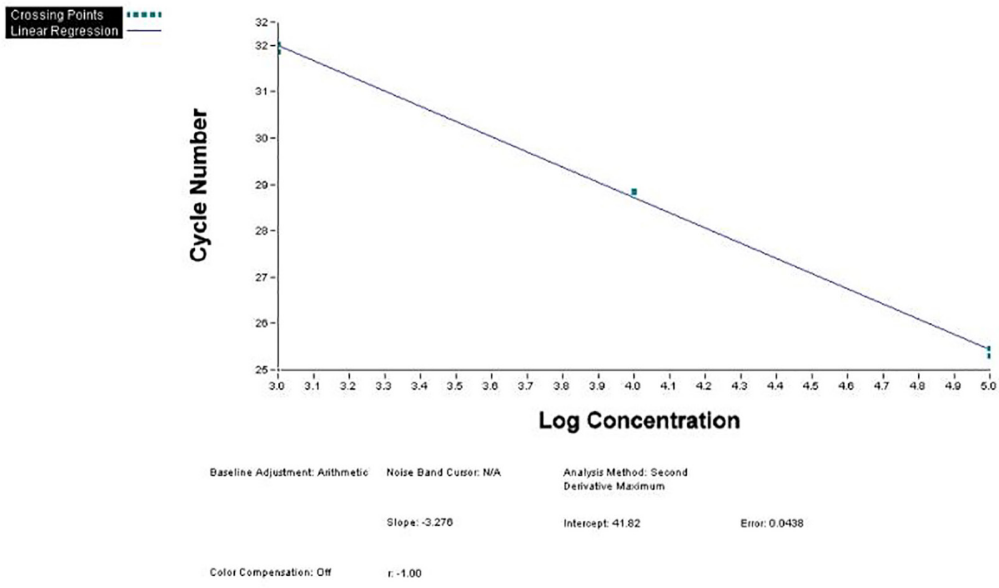

Figure 3: Standard curve for different concentrations of primers SIRT1 and ABL

For preparation of a standard diagram, the dilutions of 0.1, 0.01, and 0.001 from related cDNA to SIRT1 were prepared. The same steps were also taken for ABL primers and the curves were drawn. This step is highly important in preparation of standard diagram and optimization of the tests. After drawing the standard diagram, the slope of the diagram was calculated. Although the best range of the slope was between 3.5 and 3.6 and this slope was appropriate for calculation of the concentration of the sample, method was used in this experiment to analyze the changes in gene expression, because the slope of standard curve was equal to 3.3.

Figure 4 displays the amplification curve of real-time PCR products for primers of SIRT1 and ABL. The reactions took place in 45 cycles and the amplification curve was drawn by the machine software, based on number of cycles to intensity of fluorescence. After the end of reaction in each working run, the melting curve for aforementioned products was analyzed as well. At the end of each working run, the CT of the samples which were acquired by means of ABL and SIRT1 primers were written down.

The two groups were also compared in terms of achieving MMR based on ΔCT and the difference between the two groups was significant (figure 5).

Moreover, the two groups were compared according to ΔΔCT for SIRT1gene expression. The level of SIRT1 gene expression in the imatinib-resistant (who did not achieve MMR) group was significantly higher ($P<0.05$, figure 6).

Discussion

SIRT1 has an important role in resistance acquisition in cancers and there is an increased expression of this gene in many cancers. Previous studies showed that patients with increased expression of this gene are more likely to be resistant to many drugs, compared with patients with

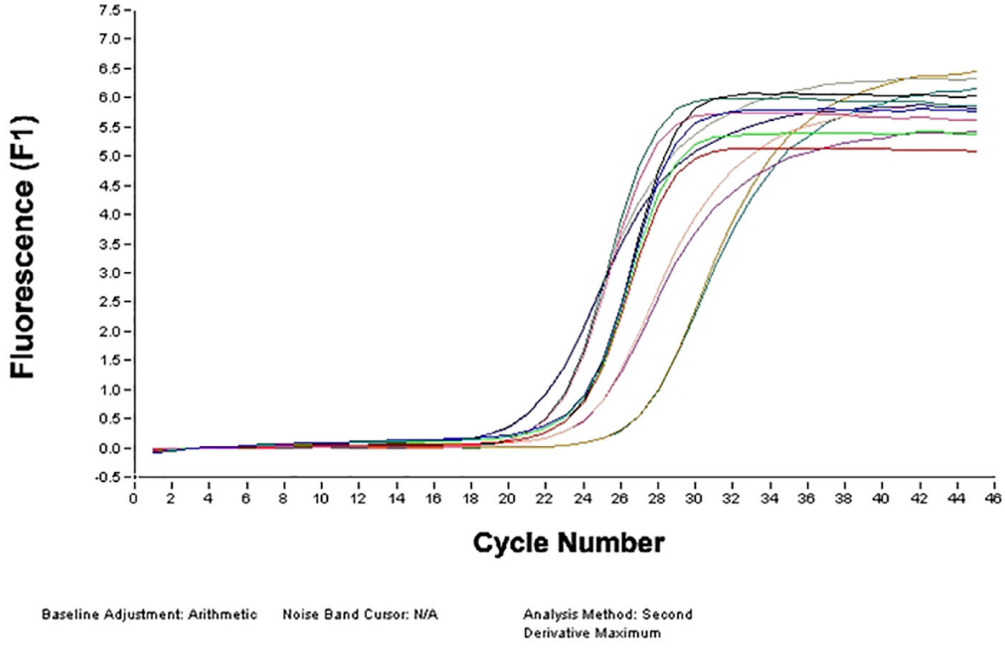

Figure 4: Amplification curve for real-time PCR products of samples obtained from the patients

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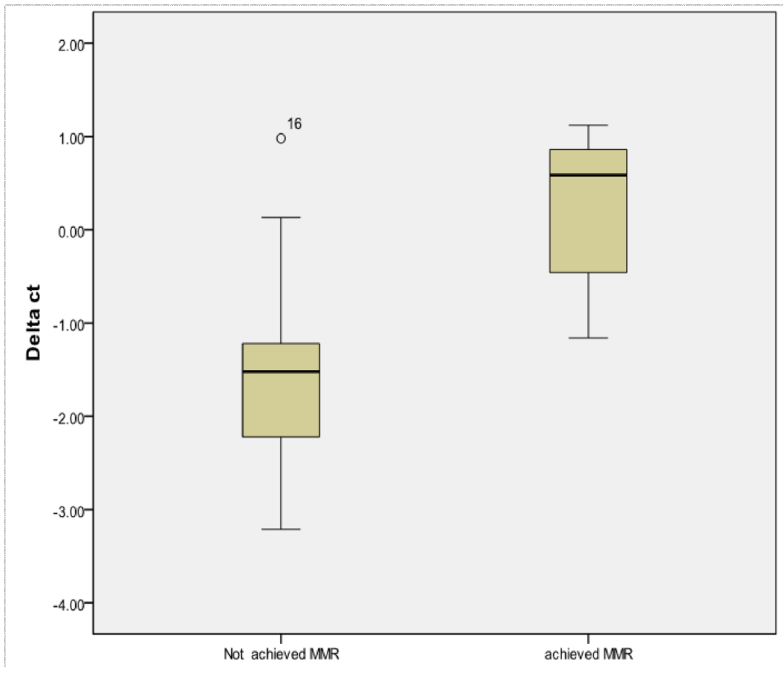

Figure 5: Comparison of two groups of patients in terms of MMR based on ΔCT (MMR= major molecular response)

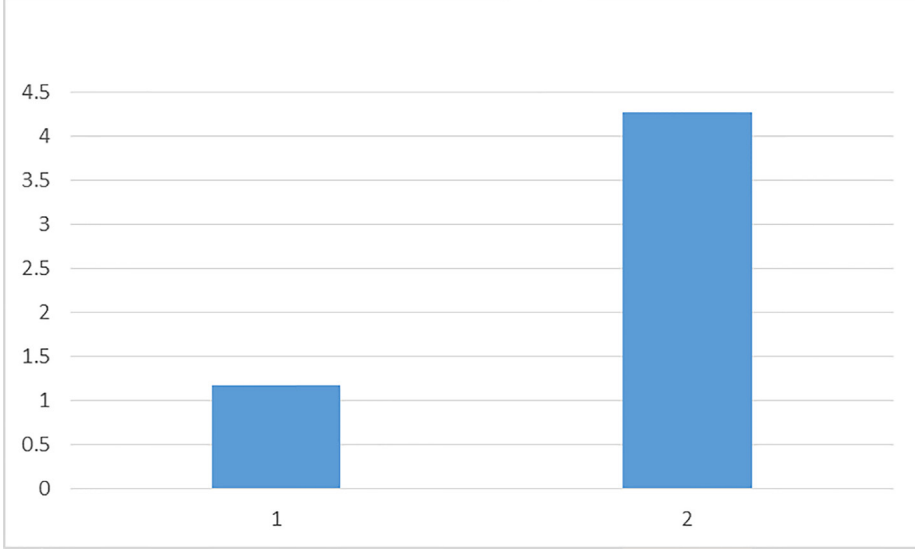

Figure 6: Comparison of the average SIRT1 gene expression in two groups of patients in terms of MMR based on ΔΔCT (1-Achived MMR; 2- Not Archived MMR)

a decreased expression of this gene.[16]

There was an increased expression of SIRT1 gene in samples from both primary chronic and blastic phases of CML cell lines. Increased expression of this gene results in acquisition of drug resistance in KCL-22 cell line.[13]

SIRT1 gene is related to different aspects of cancer such as size of the tumor and its method of development.[16] In the laboratory, blocking this gene leads to restraint of cell proliferation and cell migration. Moreover, blocking this gene leads to an increase in drug sensitivity. In animal studies the carcinogenicity and metastatic role of this gene has been confirmed. Expression of this gene has been observed in all mammalian cell lines. The product of this gene acts as a protein which plays its role in the cell nucleus. Furthermore, the expression of this gene is discussed as a factor in emergence of resistance against oxidative agents and programmed cell death. There is a controversy over the role of this gene in cancer. Increased expression of this gene has been reported in a variety of cancers, such as prostate cancer, leukemia and breast cancer. Meanwhile, there is also some role for this gene as an anti-tumor which is mentioned in some studies.[20,21]

SIRT1 gene expression is associated with advanced tumor stages and increases along with the severity of the disease. Consequently, the effects of this gene vary based on the cell type, stage of tumor progression and basic gene mutation. For example, increased expression of SIRT1 gene leads to drug resistance in liver cancers.[16]

Drug resistance in CML is in two forms; associated with the BCR-ABL and independent of BCR-ABL.[22] Role of SIRT1 in drug resistance is recently discussed in many cancers including cancer of liver, pancreas, and breast. Increased expression of this gene promotes proliferation of cancer cells and it seems this is specific to cancer cells.[23]

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Increased expression of this gene leads to up-regulation of MDR-1, a protein associated with drug resistance.[24] In some patients with CML, it was observed that this gene was responsible for deacetylation in proteins involved in DNA repair that resulted in mutations causing drug resistance. Expression of this gene not only influences over the mutation of BCR-ABL gene but also affects other genes in the carcinogenesis pathway.[25]

This study showed that imatinib-sensitive patients had a lower SIRT1 gene expression. Our results were consistent with the results of other studies on CML cell line. A distinct aspect of this study was assessment of expression of SIRT1 gene in a group of patients who were receiving imatinib mesylate for a couple of years; while previous studies were conducted on fewer patients or mostly focused on laboratory models and cell lines on culture environment.[1]

A similar study was conducted on patients afflicted with lung, liver, and prostate cancer. It was observed that patients who had an increase in SIRT1 gene expression did not respond to medications and were drug resistant.[26] Patients in our study were all in the chronic phase. SIRT1expression in a group of patients who were drug resistant had increased compared with the group who were sensitive to the drug. Likewise, in a study on KCL-22 cell line, removal of SIRT1 gene in cultivation environment led to better molecular response to treatment with imatinib mesylate.[13]

Conclusion

In our study on patients with CML, it was observed that patients with resistance to imatinib mesylate had a higher level of SIRT1 gene expression.

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Conflict of Interest: None declared.

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ORIGINAL ARTICLE

The Survey of Effective Agents on Factor VIII and IX Inhibitors in Patients with Hemophilia A and B in Kermanshah Province

Payandeh M[1], Amirifard N[2], Sadeghi E*[2], Sadeghi M[3], Choubsaz M[2], Noor Mohammadi Far F[3]

[1]Department of Hematology and Medical Oncology, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2]Cancer Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[3]Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

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*Corresponding author: Edris Sadeghi, Cancer Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
Email: Sadeghi_mkn@yahoo.com

ABSTRACT

Background: Hemophilia is the most frequent severe hereditary hemorrhagic disease due to deficiency of coagulation factors VIII (Hemophilia A) or IX (Hemophilia B) in plasma. We aimed to identify patients with hemophilia in Kermanshah, Iran and assess the incidence of inhibitors in this population and its associated factors.

Methods: This study was conducted on patients with hemophilia A and B admitted in hospitals of Kermanshah city, referred to coagulation laboratory of Kermanshah blood transfusion organization. Variables including age, sex, family history of the patients in terms of history of hemophilia and inhibitor formation, development of inhibitor in patients, age at starting the treatment, blood group, severity of hemophilia, average of factors received per month and liver disease were assessed in patients.

Results: Of 123 patients with hemophilia A, 119 (96.7%) were men. The mean±SD age of patients with hemophilia A was 25.9±15.74 years. Only five men had developed factor VIII inhibitor. Of 25 patients with hemophilia B, 24 (96%) were men with a mean±SD age of 21.7±15.71 years. Factor IX inhibitor was not detected in any patient. There was no association between incidence of inhibitors and age at the onset of the treatment, family history of hemophilia, blood group, severity of hemophilia, average of received factor per month and liver disease. However, a positive association between incidence of inhibitors and family history of inhibitors was found (P<0.05).

Conclusion: Association between family history of inhibitor and incidence of inhibitor formation in hemophilic patients was a new finding. Therefore this outcome and genetic evaluation of these for finding relevant mutations should be considered.

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Introduction

Hemophilia is the most frequent hereditary hemorrhagic disease. Hemophilia A is the most common congenital severe bleeding disorder and is the result of a deficiency in the clotting protein factor VIII.[1,2] Factor VIII (FVIII) deficiency is an X-linked recessive disorder occurring in 1 in every 5000 male births without an ethnic predominance [3], but hemophilia B is prevalent in one in every 30000 male births.[4] According to the global survey carried out by World Federation of Hemophilia, Iran was ranked as the second in the eastern Mediterranean region next to Egypt; however, the size and distribution of the Iran hemophilic population is not well known.[5] Understanding the pathophysiological mechanisms leading to the development of inhibitory anti factor VIII antibodies in patients with hemophilia A has improved considerably over the last 2 decades.[6] In patients who have developed high titers of antibodies (inhibitors) against factor concentrates, acute bleeding can be inhibited by administering bypass agents, but it is difficult to predict

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the effectiveness of such treatment in individual cases.[7] Different attempts have been suggested to overcome or eradicate against development of coagulation factor inhibitors. Immune tolerance induction (ITI) is an effective strategy often warranted in these patients.[8] A variety of mutations in the genes encoding FVIII or FIX on X chromosome is being defined that lead to non-functional proteins or their complete absence. Generally, point mutations in the F9 gene can lead to severe hemophilia B, whereas deletions or major inversions in the F8 gene lead to severe hemophilia A.[9] In Iran, most patients with hemophilia A have received several replacement therapies such as fresh frozen plasma (FFP), cryoprecipitate, and factor VIII concentrate. We aimed to determine the number of patients with inhibitor and associated factors in a population of patients with hemophilia referring to Kermanshah blood transfusion organization.

Patients and Methods

In this descriptive cross-sectional study, all patients with hemophilia referred to coagulation laboratory of Kermanshah blood transfusion organization were enrolled. Two ml blood with 9 to 1 ratio with 3.2 grams per deciliter of sodium citrate was obtained and centrifuged with speed of 2000/15 RPM for preparing of *platelet poor plasma*. Then, 0.2 ml plasma was combined with 0.2 ml of normal plasma (at least 15 samples of healthy individuals) and control sample was contained 0.2 ml normal plasma with 0.2 ml deficient factor *VIII* for hemophilia type A and 0.2 ml normal plasma with 0.2 ml deficient factor IX for hemophilia type B. Samples were kept for 2 to 4 hours to measure factor *VIII* inhibitor and 1 hour for factor IX inhibitor in a water bath at 37 °C. After this period, factor *VIII* and IX were measured with Coagulometer STAGO using a formula based on the level of coagulation factor inhibitor in Bethesda unit. Data were analyzed using descriptive statistics and analysis of T-test, Chi-square test and *Mann-Whitney U* non-parametric test with SPSS 19.

Table 1: The characteristics for hemophilia A (n=123)

Variables	n(%)	Mean±SD
Age(year)		25.9±15.7
Sex		
Male	119(71.1)	
Female	4(28.9)	
Marital Status		
Single	69(56.4)	
Married	54(43.7)	
Education Status		
Illiterate	18(14.6)	
Less than diploma	76(61.8)	
Diploma	15(12.2)	
College education	14(11.4)	
The Distribution		
Kermanshah	61(49.6)	
Songor	12(9.8)	
Other cities	50(40.6)	
Kind of Blood Type		
O^+	47(38.2)	
A^+	45(36.6)	
Other	31(25.2)	
Separation of Intensity Disease		
Mild	19(15.4)	
Moderate	30(24.4)	
Severe	74(60.2)	
Hepatic Involvement		
Positive	65(52.8)	
Negative	58(47.2)	
Treatment Start's Age(month)		
<6	18(14.6)	
6-12	25(20.3)	
>12	80(65.1)	
Family History of Hemophilia A		
Positive	75(60.1)	
Negative	48(39.9)	
Family History of Inhibitor		
Positive	5(4.9)	
Negative	118(95.1)	

Results

Out of 148 patients with hemophilia, 123 had hemophilia A and 25 had hemophilia B. Of the 123 patients with hemophilia A, 119 (96.7%) were men. The mean±SD age of the patients was 25.9±15.74 years. There were only five men with FVIII inhibitor. Female patients did not develop any FVIII inhibitor. Sixty-nine patients (56.4%) patients were single and 54(43.7%) were married. Eighteen patients (14.6%) were illiterate, 76(61.8%) were less than high school diploma, 15(12.2%) had diploma certificate and 14(11.4%) had college education. Majority of patients (49.6%) with hemophilia A were from Kermanshah city and then Songor city (9.8%). Frequency of blood groups O+ and A+ in the patients was 38.2% and 36.6%, respectively (Table 1).

Out of 25 patients with hemophilia B, 24 (96%) were men with a mean±SD age of 21.7±15.71 years. No patient developed factor IX inhibitor. Twelve patients (48%) patients were single and 13(52%) were married. Two patients (8%) were illiterate, 11(44%) were less than high school diploma, 5(20%) had diploma certificate and 7(28%) had college education. Majority of patients (60%) with hemophilia B were from Kermanshah province (Iran). Frequency of blood groups O+ and A+ in the patients was 56% and 20%, respectively (Table 2).

There was no association between incidence of inhibitors and age at onset of treatment, family history of hemophilia, blood group, severity of hemophilia, average of factors received per month and liver disease. But we found an association between development of inhibitors and family history of inhibitor formation ($P<0.05$).

Discussion

Incidence of development of inhibitors in patients with hemophilia A in different studies have been reported to be from 8.5-27%.[10-16] In our study it was 4% and all patients were men. In a study reporting hemophilia from Iran, neither of patients with hemophilia B developed inhibitor similar to our study.[10] The most and least common blood groups in our patients with inhibitor

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Table 2: The characteristics for hemophilia B (n=25)

Variables	n(%)	Mean±SD
Age(year)		21.7±15.7
Sex		
Male	24(96)	
Female	1(4)	
Marital Status		
Single	13(52)	
Married	12(48)	
Education Status		
Illiterate	2(8)	
Less than diploma	11(44)	
Diploma	5(20)	
College education	7(28)	
Kind of Blood Type		
O^+	14(56)	
A^+	5(20)	
Other	6(24)	
Separation of Intensity Disease		
Mild	7(28)	
Moderate	8(32)	
Severe	10(40)	
Hepatic Involvement		
Positive	11(44)	
Negative	14(66)	
Treatment Start's Age (month)		
<6	4(16)	
6-12	4(16)	
>12	18(68)	
Family History of Hemophilia B		
Positive	22(88)	
Negative	3(12)	
Family History of Inhibitor		
Positive	0(0)	
Negative	25(100)	

were O and AB blood groups, respectively, compatible with blood group frequency in the general population.[17] Most of our patients with hemophilia A and B had blood groups O^+ and A^+. The patient's age is generally accepted to be an important risk factor for inhibitor development.[18] There are conflicting data regarding age at first treatment as a risk factor for inhibitor formation. Two small cohort studies found an inverse association between the age (<6 months) of first exposure to factor and inhibitor formation but they were not controlled for other risk factors for inhibitor formation.[19,20] We found no association between incidence of inhibitors and age at which treatment was started. Inhibitor formation was a less common complication in patients with mild or moderate hemophilia occurring in approximately 3–13% of them.[15,21] In a comprehensive study from Iran it was indicated that there was a significant association between disease severity and inhibitor formation (P<0.0001).[10] Another study showed that overall prevalence of inhibitor formation was 14.4%, whereas its prevalence in severe hemophilia A patients was reported to be 22.8%.[14] Inhibitor activity was not detected in either of the 14 patients with mild hemophilia while it was present in 9 of 27 (33%) patients with moderate, and 7 of 17 (41%) with severe disease.[16] Incidence of Inhibitor formation in mild and moderate hemophilia was 3.5% and 9.4%, respectively. Overall, 93% of the patients with inhibitor were of patients with moderate and severe hemophilia A.[22] In a study on 1280 patients, there were 368 (28.8%), 277 (21.6%) and 635 (49.6%) patients with mild, moderate and severe hemophilia A, respectively.[13] Of 123 patients with hemophilia A in this study, 19 (15.4%), 30 (24.4%) and 74(60.2%) had mild, moderate and severe type of the disease, respectively. Patients of African or Hispanic heritage have an increased risk of inhibitor formation.[23] We could not find any association between incidence of inhibitor with family history of hemophilia in the literature but such an association between inhibitor formation and family history of inhibitor did exist in our patients, although there was a very small population of only five and needs further studies confirming this finding (P<0.05).

Conclusion

Association between family history of inhibitors and incidence of inhibitor formation was a new finding in our study. Future studies including a large number of patients are required to approve such association and then look for more genetic mutations predisposing to development of inhibitors in hemophiliac patients.

Conflict of Interest: None declared.

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Case Report

Neuroendocrine Carcinoma of the Stomach; A Case Report

Akhter R[1], Khuroo MS[2], Dar ZA[3], Qayoom N[1], Bhat N[1]

1. Senior Resident, Department of Pathology Government Medical College, Srinagar, India
2. Assistant Professor, Department of Pathology Government Medical College, Srinagar, India
3. P.G. Department of Zoology, University of Kashmir, Srinigar, India

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Stomach

*Corresponding author: Ehsan Shahverdi, Zubair Ahmad Dar, PhD;
Lecturer Zoology,
Department of Education
Srinagar-190 006, J&K, India.
Tel: 91 9469080593
Email: zubair_mun@yahoo.co.in

ABSTRACT

Gastric neuroendocrine carcinoma is a rare tumor which has a poor prognosis. Herein, we present a 55-year-old woman who presented with complaints of recurrent vomiting, hematemesis and weight loss. Endoscopic examination showed a large ulcerated mass in the antrum. Microscopic evaluation of the specimen taken through biopsy was compatible with a small round cell tumor. However, definitive histopathological diagnosis was made after surgical resection which revealed a neuroendocrine neoplasm immunohistochemically positive for Chromogranin A and Neuron specific enolase. As a result a diagnosis of neuroendocrine carcinoma of stomach was made for the patient.

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Introduction

Neuroendocrine carcinoma of stomach is a rare neoplasm with poor prognosis and accounts for less than 1% of all gastric tumors.[1,2] They can be subclassified into 3 distinct groups: Type 1 is associated with chronic atrophic gastritis/pernicious anemia (70%-80%), Type 2 is associated with Zollinger-Ellison syndrome and multiple endocrine neoplasia type 1 and Type 3 comprise sporadic neuroendocrine tumors of the stomach (15%-20%).[3] Here, we describe a case of sporadic neuroendocrine carcinoma of the stomach whose exact pathological diagnosis was made after histopathological review of surgical specimens while biopsied material could not characterize the diagnosis.

Case Presentation

A 55-year-old woman presented with a history of recurrent vomiting and hematemesis for 4-5 days and weight loss for the last 3 weeks. Hemogram was normal except for mild anemia. Computed tomography of the abdomen revealed an ill-defined soft tissue density in the gastric antrum measuring 7×5.6 cm with contiguous involvement of stomach and duodenum and obliterating fat planes between the lesion and gall bladder. Upper gastrointestinal endoscopy showed a large ulcerated mass in the antrum through which biopsy was performed and a small round cell tumor was found. A distal partial gastrectomy with gastrojejunostomy was performed. On gross examination, the specimen was measured to be 15 cm along greater curvature and 8cm along lesser curvature (figure 1 A and B). On cut section an ulcero-infiltrative tumor measuring 7×5 cm was identified in the gastric antrum along the lesser curvature.

Microscopically, the tumor was composed of round to cuboidal cells with vesicular nuclei and dispersed chromatin with tumor cells arranged in sheets forming nests at some places. The tumor was mitotically active with 6-7 mitoses/HPF and was penetrating serosa with vascular invasion. The resected margins, adjacent gastric mucosa and omental fat were histologically unremarkable.

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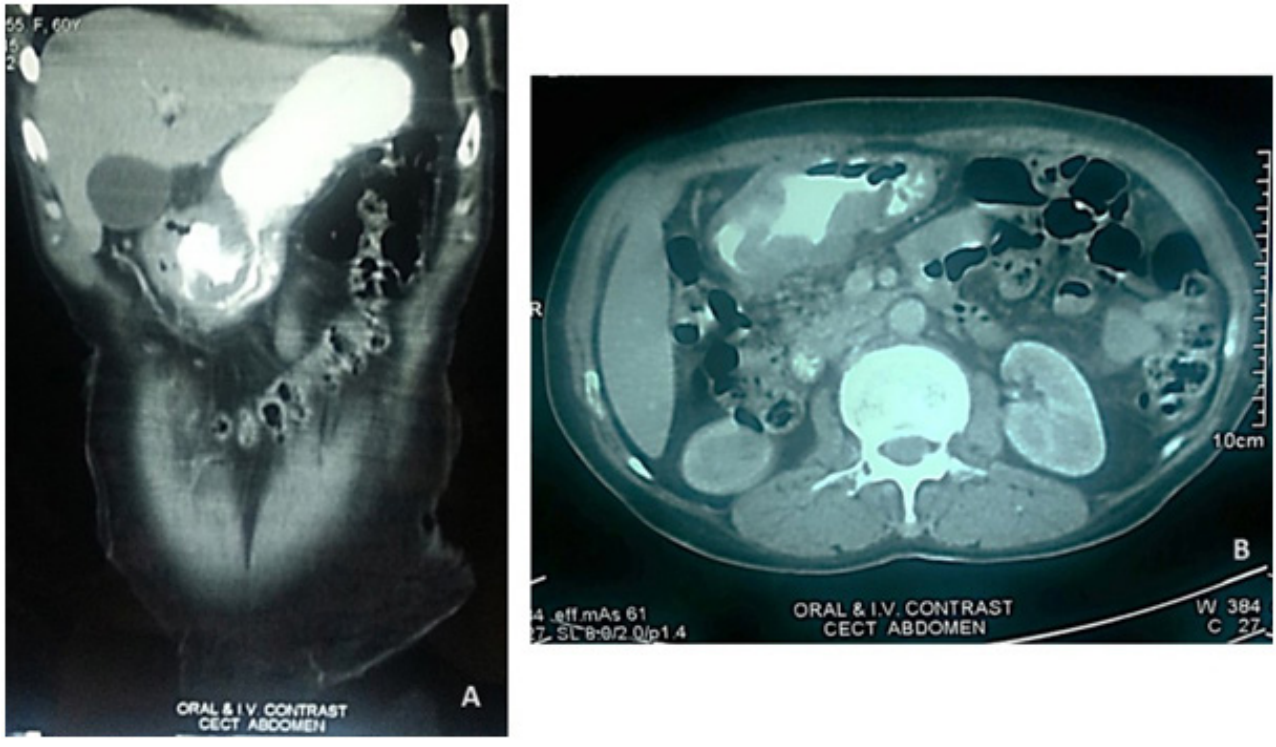

Figure 1: A&B) Contrast Enhanced Computed Tomography (CECT) of the abdomen. Well distended stomach showing circumferential thickening and mass within the gastric wall. There is peri-gastric fat infiltration and lymphadenopathy.

Out of the ten lymph nodes dissected out, four showed metastatic deposits of the tumor.

Immunohistochemically, the tumor cells were positive for Chromogranin A and neuron Specific Enolase (NSE) (figures 2 A-D). Based on histological tumor patterns, positivity for neuroendocrine markers and mitotic figures and according to recent WHO criteria, a diagnosis of neuroendocrine carcinoma of stomach Grade 3 was made. The post-operative period was uneventful and she was discharged on 10th post-operative day.

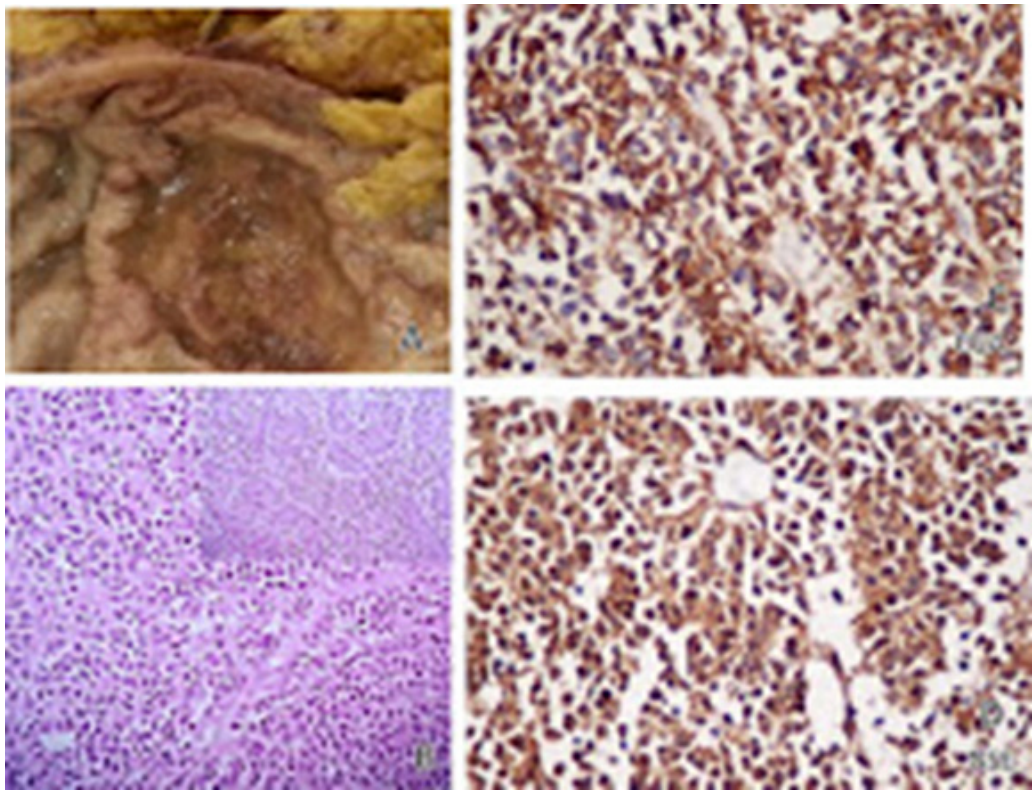

Figure 2: A) Gross photomicrograph of gastrectomy specimen showing a large ulcerated growth infiltrating in to serosa. B) (H&E 400×): Small to medium sized tumor cells with moderate to scant amount of cytoplasm. Coarse, salt-pepper chromatin with tumor cells arranged in sheets, trabeculae and rosettes (inset). C) (Chromogranin A; CGA): Tumor cells stained strongly positive for immunostain Chromogranin A. D) (Neuron specific Enolase; NSE): Tumor cells stained strongly positive for immunostain NSE.

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Discussion

Gastric neuroendocrine neoplasms (NENs) comprise a group of tumors that exhibit a spectrum of histopathological variations, ranging from clearly benign tumors to highly malignant ones. NEN is an epithelial neoplasm with predominant neuroendocrine differentiation and is an uncommon tumor with multiple sites of occurrence.[4] Neoplasms may originate from any of the endocrine cells of the gastric wall, most commonly the enterochromaffin-like cells (ECL) of the oxyntic mucosa. Proliferation of these ECL cells could result in hyperplasia, dysplasia and neoplasia.[5] The first reports of tumors with the characteristics of gastrointestinal NETs can be traced in the medical literature of the late 19th century. Lubarsch (1888)[6] is credited with the first detailed description of such tumors in autopsy material while Ranson (1890)[7] described a patient with a tumor of the terminal ileum, hepatic metastases, diarrhea and postprandial exacerbation of dyspnoea. In 1907, Oberndorfer coined the term "carcinoid" (Karzinoid) to distinguish the more benign course of these rare tumors from that of the much commoner adenocarcinomas.[8] Askanazy reported the first two cases of gastric NET in 1923.[9] Christodoulopoulos and Klotz reported 79 cases of carcinoid tumor of stomach mainly diagnosed at autopsy.[10] Neuroendocrine carcinoma of stomach is a rare neoplasm with poor prognosis and accounts for less than 1% of all gastric tumors.[1,2] It occurs mostly in adults, has a prelidiction for females and is rare in children.[11]

Recently WHO classified the gastric neuroendocrine carcinoma to well differentiated neuroendocrine tumor, well differentiated carcinoma and poorly differentiated carcinoma based on the biological behavior, tumor size, tumor infiltration and angioinvasion.[12]

The proliferative rate of the tumor is assessed based on number of mitoses/10 HPF or the percentage of neoplastic cells immunolabelleling for Ki-67, a proliferative marker.[12] Also lymph node involvement is a significant predictor of survival, because the lymph node involvement is an important indicator in the Tumor Node Metastasis (TNM) staging of gastric NENs.[13] In present case, non-Hodgkin's lymphoma and poorly differentiated carcinoma were diagnosed on tumor specimen taken through endoscopic biopsy whereas subsequently a diagnosis of neuroendocrine carcinoma was made based on tumor patterns such as nesting, typical neuroendocrine chromatin and mitotic count of >50 mitoses/10HPF considering recent WHO criteria of neuroendocrine neoplasms.

Our diagnosis was further supported by positivity of tumor cells for chromogranin and NSE stains.

Conclusion

Although a rare tumor, neuroendocrine carcinoma should be considered a potential diagnosis in endoscopic biopsies. An early diagnosis and appropriate treatment can be instituted before lymphatic spread and dissemination is supervened.

Conflict of Interest: None declared.

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Case Report

Autoimmune Lymphoproliferative Syndrome Misdiagnosed as Hemophagocytic Lymphohistiocytosis; A Case Report

Abolghasemi H[1], Shahverdi E*[2], Dolatimehr F[2], Oghli RM[2]

1. Department of Pediatrics, Baqiyatallah University of Medical Sciences, Tehran, Iran
2. Students' Research Committee (SRC), Baqiyatallah University of Medical Sciences, Tehran, Iran

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*Corresponding author: Ehsan Shahverdi, MD; Baqiyatallah University of Medical Sciences, Mollasadra st., Vanaq sq., Tehran, Iran
Tel/Fax: +98 21 88620826
Email: shahverdi_ehsan@yahoo.com

ABSTRACT

Autoimmune lymphoproliferative Syndrome (ALPS) is a rare inherited disorder of apoptosis. It usually presents with chronic lymphadenopathy, splenomegaly, and symptomatic cytopenia in a child. Herein, we report a 14-year-old boy with symptoms misdiagnosed as hemophagocytic lymphohistiocytosis who was treated before ALPS was diagnosed for the patient. This case should alert pediatricians to consider ALPS in differential diagnosis of a child with lymphadenopathy, splenomegaly, and cytopenia.

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Introduction

Autoimmune lymphoproliferative syndrome (ALPS) is a rare disease leading to cytopenia. Lymphoproliferative disorders, such as ALPS and hemophagocytic lymphohistiocytosis (HLH) both could be considered as differential diagnosis for lymphadenopathy, splenomegaly, and pancytopenia. Tables 1 and 2 show the diagnostic criteria for ALPS and HLH, respectively.[1,2]

Patients with ALPS have nonmalignant lymphadenopathy with/without splenomegaly associated with an increased $CD3^+$, TCR α/β^+ lymphocytes that do not express CD4 or CD8, referred to as double-negative T cells (DNTs). Patients may also have elevated levels of vitamin B-12.[1]

Herein, we describe a 14-year-old boy who was diagnosed with ALPS following a presumptive diagnosis of HLH.

Case Presentation

A 14-year-old boy was admitted with a history of seven months of abdominal pain and persistent fever. His medical history was unremarkable. Vital signs at admission were: temperature 38.8 °C, pulse rate: 90 beats per minute and blood pressure 123/70 mmHg. Palpable cervical and axillary lymphadenopathy was noted on physical examination. The patient's abdomen was diffusely tender to palpation mainly on the left upper quadrant. The spleen and liver were palpable 5 cm and 4 cm below costal margin, respectively.

Laboratory tests revealed WBC: 1300/mm^3, 40% polymorphonuclear leukocytes, 43.6% lymphocytes, Hb: 6.9 g/dl, Hct: 28%, Platelet: 57000 /mm^3. All biochemistry tests were within normal range. LDH: 712 IU/l (normal: 207 - 414 IU/l), Direct bilirubin: 0.9 mg/dl.

Wright agglutination test, coombs test, Rheumatoid

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Table 1: Diagnostic Criteria for ALPS

Required criteria
Chronic (>6mo), nonmalignant, noninfectious lymphadenopathy and/or splenomegaly
Elevated $CD3^+TCRab^+CD4^-CD8^-$ DNTs (>1.5% of total lymphocytes or >2.5% of CD3+ lymphocytes) in the setting of normal or elevated lymphocyte counts
Accessory criteria
Primary
Defective lymphocyte apoptosis
Somatic or germ-line pathogenic mutation in FAS, FASLG, or CASP10
Secondary
Elevated plasma sFASL levels (>200 pg/mL), plasma interleukin (IL) -10 levels (>20 pg/mL)
Serum or plasma vitamin B-12 levels (>1500 pg/mL) or plasma IL-18 levels >500 pg/mL
Typical immunohistologic findings
Autoimmune cytopenias (hemolytic anemia, thrombocytopenia, or neutropenia)
Elevated IgG levels (polyclonal hypergammaglobulinemia)
Family history of a nonmalignant/noninfectious lymphoproliferation with or without autoimmunity
Definitive diagnosis: both required criteria plus 1 primary accessory criterion
Probable diagnosis: both required criteria plus 1 secondary accessory criterion

Reprinted with permission from Oliveira JB, Bleesing JJ, Dianzani U, et al. Blood. 2010;116(14):e35–e40. IgG, immunoglobulin G; sFASL, Souble FasLigand.

Table 2: Diagnostic Criteria for HLH

(A) A molecular diagnosis consistent with HLH: pathologic mutations of PRF1, UNC13D, Munc18-2, Rab27a, STX11, SH2D1A, or BIRC4 Or (B) If 5 of the 8 criteria listed below are fulfilled:
1. Fever>38.5°C
2. Splenomegaly
3. Cytopenias (affecting at least 2 of 3 lineages in the peripheral blood) Hemoglobin <9 g/dL (in infants <4 weeks: hemoglobin <0 g/dL) Platelets $<100 \times 10^3$ per mL Neutrophils $<1\times10^3$ per mL
4. Hypertriglyceridemia (fasting: >265 mg/dL) and/or hypofibrinogenemia (<150 mg/dL)
5. Hemophagocytosis in bone marrow, spleen, lymph nodes, or liver
6. Low or absent NK-cell activity
7. Ferritin >500 ng/mL
8. Elevated sCD25 (a-chain of sIL-2 receptor)

Reprinted with permission from Jordan MB, Allen CE, Weitzman Sheila, et al. Blood. 2011;118:4041–4052. NK, natural killer; sCD25, Soluble CD25 (a term sometimes used for alpha- chain of soluble IL-2 receptor).

factor and CRP were negative. Immunoelectrophoresis demonstrated Ig M: 290 mg/dl (normal : 40-230 mg/dl) and Ig G: 3300 mg/dl (normal : 700-1600 mg/dl). ANA, Anti double-stranded DNA (anti-dsDNA), Anti-citrulline antibodies, CH_{50}, C-ANCA, P-ANCA, Anti Toxoplasma (IgM and IgG), Anti Brucella (Ig M and Ig G), IFA for visceral leishmaniasis, Anti body for Borrellia and plasmodium malaria, viral hepatitis and HIV serology tests were negative. Flowcytometry revealed CD_3: 185mg/dl (normal: 90-180 mg/dl), CD_4: 35 mg/dl (normal: 10-40 mg/dl) and CD_8: 0. Anti EBV-VCA Ig M was 16 U/ml(normal<20 U/ml).

Double negative (DN) T cells were positive. He also showed hypofibrinogenemia with plasma fibrinogen of 150 mg/dl (normal: 180-540 mg/dl). Serum vitamin B12 level was in normal range. PCR for Cytomegalovirus (CMV)-DNA and Herpes simplex virus (HSV)-DNA was negative. A diagnosis of HLH was considered since he met four of 8 criteria including pancytopenia, >38.5 °C fever, splenomegaly and hypofibrinogenemia. Notably, he had normal ferritin levels.

One month later, a repeated ferritin measurement was 876 ng/ml, providing the fifth criterion for the diagnosis of HLH. Treatment of HLH was initiated according to the HLH-2004 protocol.[3] Because of huge splenomegaly and discomfort of the patient, splenectomy was performed for therapeutic and diagnostic purposes where the spleen was found to have diameters of 28×17×5 cm and weight of 1900 gr. Histopathology revealed hypersplenism with widened splenic cords and rare to absent hemophagocytosis. It was also noted that the spleen had atypical T-cell hyperplasia with DNTs, consistent with the diagnosis of ALPS.

He was then treated with prednisone 15 mg once daily and azathioprine 150 mg per day accordingly. Clinical and hematologic remission was ensued so that treatment was continued while tapering off the immunosuppressives. The patient is in good clinical conditions seven months after treatment.

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Discussion

ALPS and HLH are both considered in differential diagnosis of lymphadenopathy, splenomegaly, and pancytopenia and have many clinical and laboratory features in common which could be overlapping. Spergel and colleagues reported a 6-year-old girl with ALPS after initial diagnosis of HLH.[4] Both present in childhood and are characterized by lymphadenopathy, splenomegaly along with evidence of immune dysregulation such as cytopenia.[5] However, distinguishing between ALPS and HLH is vital because of different therapeutic measures.

Patients with ALPS often require long-term immunosuppressive therapies with corticosteroids and also steroid-sparing measures.[6] They also need to be taken care of for development of possible hematologic malignancies specially Hodgkin and non-Hodgkin's lymphoma.[7] Splenectomy is not necessary either for diagnosis or management in most of the cases.[6,8,9]

In contrast, the standard treatment of HLH is chemotherapy, including dexamethasone and cyclosporine with or without etoposide and if the patient has a family history of HLH and/or has central nervous system disease, allogeneic bone marrow transplantation is indicated.[2] Considering that none of these circumstances existed in our patient, bone marrow transplantation was not considered for our patient.

In this case, the initial diagnosis of HLH caused a delay in identification of the actual diagnosis for almost one month. He underwent splenectomy before a definitive diagnosis of ALPS was established, that might be an additional risk factor in the future for mounting probable infections in a patient who is also receiving immunosuppressive treatments. As a result, there is a need to carefully monitor the patient in the long term for the risk of pneumococcal sepsis secondary to splenectomy.[10]

In order to differentiate ALPS from HLH, it is important to be careful about diagnostic criteria of both conditions. Elevated serum biomarkers (vitamin B-12, sFASL, IL-10, IL-18) with evidence of autoimmune cytopenia can point toward a diagnosis of ALPS. The level of serum vitamin B12 >1.5 picograms/L bears important diagnostic significance.[5] However, our case had normal level of vitamin B12. Some recent studies have shown elevated IL-10 levels in HLH patients.[11,12]

Serum ferritin level could be of help to differentiate ALPS from HLH. Its level in ALPS is generally lower than 3000 ng/mL, which is reportedly specific for HLH.[13]

Conclusion

We have highlighted this case to emphasize the necessity of considering rare disorders, particularly ALPS in differential diagnosis of patients presenting with lymphadenopathy, splenomegaly and cytopenia.

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Letter to Editor

Is Colorectal Cancer an Unusual Malignancy among Iranian Children?

Karimi M[1], Shahriari M[2], Abdolkarimi B*[3], Mokhtari M[4]

1. Professor of Pediatric Hematology-Oncology, Department of Pediatrics, Shiraz University of Medical Sciences, Shiraz, Iran
2. Associate Professor of Pediatric Hematology-Oncology, Department of Pediatrics, Shiraz University of Medical Sciences, Shiraz, Iran
3. Fellowship of Pediatric Hematology-Oncology, Shiraz University of Medical Sciences, Shiraz, Iran
4. Assistant Professor of pathology, Department of Pathology, Shiraz University of Medical Sciences, Shiraz, Iran

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*Corresponding author: Abdolkarimi B,
Email: b.abdolkarimi@yahoo.com

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Dear Editors

Adenocarcinoma of colon and rectum is the second most common cancer of the gastrointestinal (GI) tract in children. The development of carcinoma of colon in general appears to be associated with several predisposing factors such as familial polyposis, hereditary non-polyposis syndromes, ulcerative colitis, previous ureterosigmoidostomy or radiation therapy and dietary factors (high fat or low fiber diets) [1]. Here we report three adolescents with colorectal cancer referring to Amir Oncology Hospital, Shiraz, southern Iran, presenting with various signs and symptoms including acute abdominal pain, painless rectorrhagia, and refractory iron deficiency anemia. They did not have any known predisposing risk factor.

Patient 1 was a 14-year-old girl presented with acute abdomen. Abdominal sonography showed a target-like lesion on the thickened segmental bowel wall with a protrusion of the serosa which was surrounded by localized ascites in the lower abdomen. She was found to have a right-sided colon cancer at laparotomy. Histology showed stage 4 Duck. She was diagnosed to have a brain metastasis. Patient 2 was a 16 year-old boy presented with refractory iron deficiency anemia due to metastatic colorectal cancer without any underlying disease in the GI tract. The patient was treated by large amounts of iron supplement and was referred for evaluation of refractory iron deficiency anemia. Patient 3 was a 12 year-old girl presented with painless rectorrhagia without any abdominal complaints. Colonoscopic study revealed typical colon lesions in sigmoid, descending the colon and rectum. Family history was unremarkable for adenomatous polyps.

Symptoms of colon cancer in children are nonspecific and include chronic persistent abdominal pain (90%), emesis, bowel habit changes, weight loss (77%), occult blood in the stool with chronic anemia (60%), tenesmus,[2] and a palpable abdominal mass. Therefore early diagnosis of patients without predisposing factors is associated with better outcome and prevention of advanced stages and increased rate of successful treatment modalities such as adjuvant chemotherapy after primary surgery.

Although this tumor is rare in children, physicians should be alert about the cardinal signs and symptoms and to improve patient's outcome a high index of suspicion should be kept in mind. Likewise, infrequent signs and symptoms such as acute abdomen or refractory iron deficiency should increase suspicion. Primary diagnostic modalities such as fecal occult blood, complete blood count, abdominal ultrasound and/or invasive procedures such as colonoscopy should be carefully performed in children presenting with red flags for colon cancer including lower GI bleeding, acute abdomen, or iron deficiency anemia. Moreover,

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monitoring of carcinoembryonic antigen (CEA) levels is recommended during postoperative follow-up in pediatric colon cancers similar to adults [3].

Another approach for early detection of this cancer in absence of red flags is routine screening in children predisposed to colorectal cancer as a way to increase overall prognosis. Stools may be tested or a barium enema, colonoscopy, sigmoidoscopy or virtual colonoscopy may be performed. Regardless of any test, a laboratory analysis of tissue ultimately determines existence of tumor. Therefore, cell biopsy, fluid or tissue in the colon needs to be examined to determine presence of tumor.

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