IRANIAN JOURNAL OF BLOOD AND CANCER

The Official Journal of

Iranian Pediatric Hematology and Oncology Society (IPHOS)

Volume 7, Number 5, December 2015 ISSN: 2008-4595

"Iranian Journal of Blood and Cancer" is published by "Iranian Pediatric Hematology and Oncology Society (IPHOS)" in collaboration with "Iranian Blood Transfusion Organization (IBTO)"

"IJBC" is approved as an "Academic Research Journal" by Medical Journal Commissions of the "Ministry of Health" and Medical Education of Islamic Republic of Iran".

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Iranian Journal of Blood and Cancer
Volume 7, Number 5, December 2015

IJBC 2015; 7(5): 203-205

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

EDITORIAL

Bone Involvement in Neuroblastoma

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ARTICLE INFO

Article History:
Received: 03.08.2015
Accepted: 20.10.2015

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Please cite this article as: Malek F, Alavi S. Bone Involvement in Neuroblastoma. IJBC 2015; 7(5): 203-205.

Neuroblastic tumors (ie, neuroblastoma, ganglioneuroblastoma, ganglioneuroma) are the most common extracranial solid tumors in children.[1] Neuroblastoma (NB) accounts for almost 8% of childhood malignancies. Its prognosis is extensively variable, ranging from spontaneous regression to fatal disease in spite of receiving multimodality therapy.[1] Screening programs of infants show that many cases escape detection because of spontaneous regression or maturation into benign lesions. Derivation from precursors of the sympathetic nervous system accounts for (a) primary sites in adrenal glands and in paraspinal locations from neck to pelvis and (b) high urinary levels of catecholamines in 90% of cases.[2]

This embryonal neoplasm frequently invades vascular structures and usually presents with substantial metastatic disease in bone, bone marrow, lymph nodes and liver; spread to brain is observed and lung metastasis has been reported very rarely to a maximum of 3.6%.[3] Hence, defining disease extension and precise staging requires imaging studies such as computerized tomography scans (or MRI), bone scan, metaiodobenzylguanidine (MIBG) scan, bone marrow (BM) examinations and biopsy and urine catecholamine measurements.[4]

Multiple imaging and clinical tests are needed to accurately assess patient risk with risk groups based on disease stage, patient age, and biological tumor markers.[1-4]

Around 60% of patients with NB have metastatic disease at diagnosis mostly involving bone marrow or cortical bones.[1,2]

Since the spread of tumor cells to the BM is a dismal prognostic sign for patients with NB, obviously searching for BM infiltration is of the most prominence for both staging and therapeutic purposes.[5]

Due to the International Neuroblastoma Staging System, the presence of metastasis in BM is assessed by morphological study of BM smears and trephine biopsies.[4,5] Bone involvement is detected in 55– 68% of patients who have metastatic disease at diagnosis.[5,6] Bone lesions affected by metastatic tumor cells are conventionally divided into two categories ; osteolytic and osteoblastic.[6]

Main contributors in osteolytic lesions are osteoclast activating factors such as parathyroid hormone-related protein (PTHrP) which stimulates osteoclast maturation and in osteoblastic lesions there are factors that stimulate osteoblast proliferation, differentiation and bone formation.[7] Regularly, both osteoclastic and osteoblastic processes are observed simultaneously. Hence, osteoclast inhibitors like bisphosphonate compounds have begun to be used and demonstrate encouraging results.[7,8]

The mechanisms involved in the formation of bone metastasis in NB have now begun to be elucidated. It

is documented that, as observed in breast cancer and multiple myeloma, NB bone metastases are predominantly osteolytic.[8] Sohara et al. exploiting a model of bone invasion in immunodeficient mice, revealed that NB cells recruited osteoclasts to produce osteolytic lesions and invade the bone matrix.[9] So in support of a causative role for osteoclasts in NB bone invasion, they used treatment with the bisphosphonate compound; ibandronate, which showed a dramatic delay in the progression of osteolytic lesions.[9] The existing data strongly suggest that bisphosphonates may be clinically effective in the treatment of bone metastases in neuroblastoma.[9]

Promising outcomes of trials in the animal models of bone metastasis and invasion in NB terminated to the allowance for the testing of novel therapeutic pathways more particularly focused towards bone metastasis in human neuroblastoma.[10]

A first target is osteoclasts since it has now been clearly clarified that bone metastasis in neuroblastoma is primarily an osteolytic process associated with an increased activation of osteoclasts. Over the last decade several inhibitors of osteoclast activation have been developed.[9,10]

Proper staging and monitoring of patients with neuroblastoma is profoundly dependent on scintigraphic studies. Tc-99m-MDP scan has long been known to be superior to skeletal survey for detecting metastases in cortical bone.[11]

Functional imaging with 123I-MIBG scintigraphy is a crucial tool in patients with NB both for initial staging and also response to therapy, allowing visualization of the primary tumor and metastatic lesions in the various sites including the bones.[11,12]

Various radiopharmaceuticals for positron emission tomography (PET) such as fluorine-18-fluorodeoxyglucose (18 F-FDG), fluorine-18-dihydroxyphenylalanine (18 F-DOPA), 68Ga-labelled somatostatin analogues, 11C-hydroxyephedrine (11C-HED) and 124I-MIBG are currently under investigation.[12]

Treatment protocols for NB are stratified according to risk which is outlined on the basis of biologic and clinical prognostic factors. Treatment of high-risk NB continues to be a challenge, with a high rate of relapse in the bone and bone marrow.[11,12]

More to add, high quality Tc99m-MDP bone scan images are required if the skeletal metastases of neuroblastoma, which commonly develop in the metaphyses of long bones, are to be detected.[13] Meanwhile, it plays a crucial role in follow-ups of neuroblastoma in children who present with bone involvement initially.[12,13]

Skeletal involvement in neuroblastoma can be focal or diffuse and sometimes bilaterally symmetrical. These abnormalities can be recognized on MDP bone scan only with expertise and meticulous attention to technical details.[9,13]

These alterations which have been traced on MDP bone scan during follow-ups are beneficial in evaluating the effects of the initial treatment as well as further modification of the treatment plan.[14] Peng et al. demonstrated that zoledronic acid has a dual antiosteoclastic and antitumoral activity. The data emphasize that bisphosphonate in combination with cytotoxic chemotherapy in mice with established osteolytic lesions ended in preventing bone degradation and also extending survival, so could be trialed in children with neuroblastoma that has metastasized to the bone.[8]

Conflict of Interest: None declared.

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IJBC 2015; 7(5): 206-212

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

REVIEW ARTICLE

Treatment of Newly Diagnosed and Relapsed Hodgkin Lymphoma in Children

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ARTICLE INFO

Article History:
Received: 01.06.2015
Accepted: 13.09.2015

Keywords:
Diagnosed
Treatment
Children

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ABSTRACT

Hodgkin lymphoma (HL) accounts for about 10% of all childhood cancers. Five-year survival rates with modern therapies are now approaching >90-95% as a consequence of its significant sensitivity to both chemotherapy and radiation. The current challenge is to determine how much therapy is needed to improve survival and how to adapt treatment to the patient to prevent these long term toxicities. This challenge has resulted in the development of different strategies aimed at recognizing the optimal balance between preserving overall survival and avoidance of long-term treatment-related morbidity. Strategies in children could be quite different from those used for adults with HL. More defined risk stratification through imaging studies and biologic markers have been developped. Increased knowledge of the biology of HL have led to the introduction of targeted therapies in both the frontline and relapsed patients. Collaborative multicenter studies are required to develop new combination therapies for children with newly diagnosed and refractory HL.

Please cite this article as: Oberlin O, Patte C. Treatment of Newly Diagnosed and Relapsed Hodgkin Lymphoma In Children. IJBC 2015; 7(5): 206-212.

Introduction

Hodgkin lymphoma (HL) accounts for about 10% of all childhood cancers. Five-year survival rates with modern therapies are now approaching >90-95% as a consequence of its significant sensitivity to both chemotherapy and radiation. However, the risks of late effects associated with radiation and chemotherapy including infertility, second cancers and cardiac deaths have become more widely recognized. Treatment decisions are increasingly based on minimizing late effects and long-term treatment related mortality.[1] The current challenge is to determine how much therapy is needed to ensure survival and how to tailor treatment to the individual to prevent these long term toxicities.

Newly diagnosed Hodgkin Lymphoma

The use of systemic combined chemotherapy in children allowed physicians to successfully reduce the radiation fields from total nodal irradiation (used in the 70s) to involved fields (involved and adjacent nodes) and since the last decade to involved nodes only. Simultaneously, doses were progressively reduced from 40 Gy to 20 and even 15 Gy without sacrificing clinical outcomes.[2,3]

Classical Approach

Until recently, the treatment plan (type and duration of chemotherapy, doses and fields of radiation) was determined by initial stage of the disease with some modifications only if a complete response had not been achieved by the end of the treatment regimen. To minimize exposure of all children to high cumulative doses of anthracyclines and exposure of boys to high dose of alkylating agents, a variety of combination chemotherapy protocols with efficacy equivalent to that of the original MOPP regimen were invented with increased number of agents and limiting cumulative dose of individual agents (table 1). Combination regimens include ABVD, ABVD alternating with MOPP[2], COPP/ABV[4], VAMP[3], VBVP[5], ABVE-PC[6], OPPA or OEPA plus COPP[7]. Protocols in Europe and America using these chemotherapy regimens

Table 1: Combination chemotherapy regimens Acronym Agents
ABVD: Doxorubicin, bleomycin, vinblastine, dacarbazine
AV-PC: Doxorubicin, vincristine, prednisone, cyclophosphamide
COPP/ABV: Cyclophosphamide, vincristine, procarbazine, and prednisone, alternating with doxorubicin, bleomycin, and vinblastine in a single course
MOPP: Nitrogen mustard, vincristine, procarbazine, prednisone
OEPA: Vincristine, etoposide, prednisone, doxorubicin OPPA Vincristine, procarbazine, prednisone, doxorubicin VAMP Vinblastine, doxorubicin, methotrexate, prednisone VBVP Vinblastine, bleomycin, etoposide, prednisone VEEP Vincristine, etoposide, epirubicin, prednisone
ABVE-PC: Adriamycin, bleomycin, vincristine, etoposide, prednisone, cyclophosphamide

in combination with IFRT have demonstrated event-free survival (EFS) rates greater than 90%.

Response-Based Approaches

Response based approaches have now become the standard approach in pediatric HL and decisions are taken according to the response to chemotherapy.

Decisions Regarding Radiation Therapy

The First HL trials in pediatrics relied on complete response (CR) to determine the need for radiation in addition to chemotherapy. The Children's Cancer Group (CCG)[4] and the German Pediatric Hodgkin's Disease Study Group (GPOH-HD)[7] both used CR assessment at the end of therapy to either randomly assign patients to radiation versus no radiation (CCG) or to eliminate radiation (GPOH).

The chemotherapy protocol included four to six cycles of COPP/ABV with intensified cytarabine and etoposide for stage IV disease on the CCG study. The CCG study was halted because of increased risk of relapse in non-radiated patients versus radiated patients (3-year event-free survival 87% versus 92%; P<0.057). However, these results suggested that disease control may be achieved without radiation in 85%-90% of HL patients and the overall survival was similar for both radiated and non-radiated children.[4]

The GPOH-HD 95 multicenter trial used two to six cycles of OPPA (adriamycin, vincristine, procarbazine, and prednisone)/COPP for girls and OEPA (adriamycin, vincristine, etoposide, and prednisone)/COPP for boys, with radiation dose determined by end-of-chemotherapy response. Overall, the results were excellent, but EFS was higher among intermediate (TG2) and high-risk (TG3) patients treated with involved field radiation therapy (IFRT) versus those who did not receive IFRT.[7]

Decisions Regarding the Intensity of Chemotherapy

The French Society of Pediatric Oncology study (MDH90) addressed the question of intensification of chemotherapy in early slow responder patients and dose of radiation therapy in late slow responder patients. In that study, 202 children with stage I or II HL were treated with four cycles of vinblastine, bleomycin, etoposide, prednisone (VBVP). Good responders (85% of the patients) received only four cycles of VBVP and 20 Gy IFRT alone. They did not receive anthracyclines or alkylating agents and so had a low risk of long-term gonadal toxicity. Poor responders were given an additional one to two cycles of OPPA and then either 20 Gy IFRT (good responders at second evaluation) or 40 Gy IFRT (poor responders). The 5-year OS and EFS were 97.5% and 91.1%, respectively.[5]

The COG study P9425 was based on early response to limit cumulative chemotherapy in advanced-stage HL patients and to enhance early response through a dose-dense chemotherapy regimen (adriamycin, bleomycin, vincristine, etoposide, prednisone, cyclophosphamide (ABVE-PC). Early response, defined by gallium scan and computed tomography (CT; 50% 2-dimensional reduction), was evaluated after three cycles (9 weeks) of chemotherapy. Rapid early response (RER) was documented in 63% of patients who proceeded to 21 Gy IFRT. Slow early responders (SER) received two additional courses of ABVE-PC, followed by 21 Gy IFRT. Five-year EFS and OS were 84% and 95%, respectively. No difference in outcome was distinguishable for "rapid early response" group versus "slow early response" group (86% vs. 83%; P<0.85), thus achieving the goal of delivering optimal therapy titrated to the need of an individual within the context of a clinical trial.[6]

Implementation of the Early Response to FDG-PET Scan for Therapeutic Decision

The 18F-fluorodeoxyglucose positron emission tomography with computerized tomography (FDG-PET/CT) is a functional imaging modality that has become a standard tool complementing contrast enhanced CT (CECT) scans in the diagnosis and management of HL. Evaluation of the disease with FDG-PET scan before chemotherapy identifies the correct pretreatment stage in HL more accurately compared with CECT. One study of patients with early-stage HL showed that 36% of lymph nodes detected by PET were radiographically occult on CT scan.[8] However, FDG-PET/CT is reported to upstage the disease from early to advanced stage in only 10–15% of patients in whom treatment is ultimately modified.[9,10]

FDG-PET scan has showed the prognostic value of early response in treatment of HL. In a prospective study of 88 patients with early-stage non bulky HL uniformly treated with a chemotherapy regimen of AVG (doxorubicin, vinblastine, gemcitabine), 2-year PFS rates were 88% and 54% for negative and positive FDG-PET groups, respectively (P=0.0009).[11]

The European pediatric (EuroNet-PHL-C1, figure 1) has tried a procarbazine-free protocol and tailored treatment

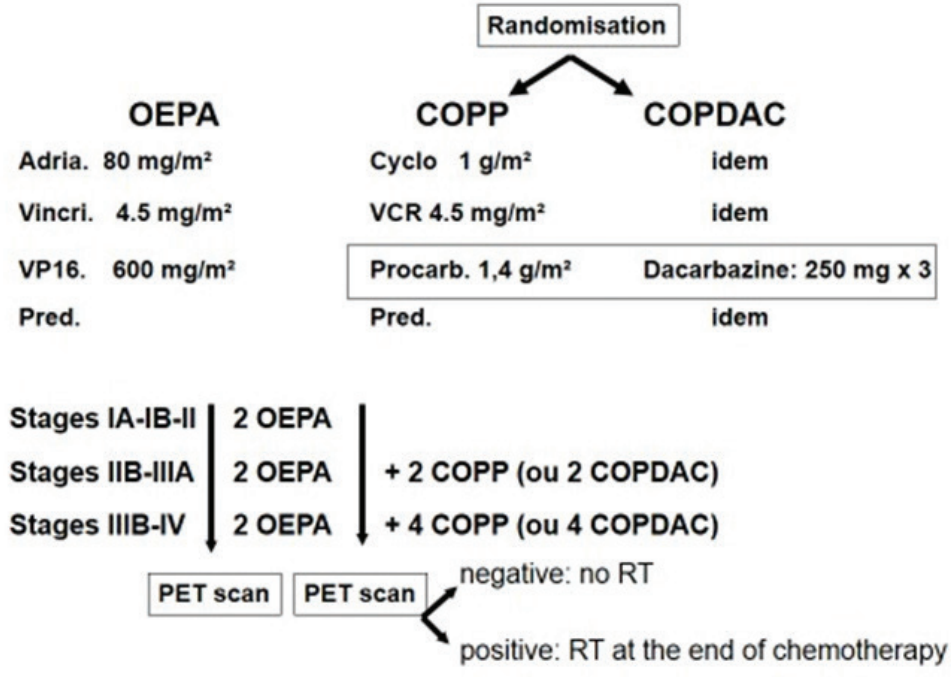

Figure 1: First European study EuroNet-PHL-C1
17-AAG=Geldanamycin; AKT=AKT kinase; ERK=Extracellular signal regulated kinase; HDACs=Histone deacetylases; HRS=Hodgkin and Reed-Sternberg cell; JAK=Janus kinases; MMAE=Monomethylauristatin E; mTOR=Mammalian target of rapamycin; NF-KB=Nuclear factor kappa B; STAT=Signal transducer and activator of transcription

according to the early metabolic response (depicted by FDG-PET scan after two courses of chemotherapy) attempting to minimize the proportion of children receiving radiation therapy. Patients with an adequate response after 2 OEPA courses (roughly: CR or PR but PET-negative) were not irradiated, whatever the treatment groups. Patients with higher stages than IIA were randomly assigned to receive either the standard COPP protocol or COPDAC in which dacarbazine replaces Procarbazine. By October 2012, 2033 patients had been registered into the trial of whom 1593 were eligible for the third interim analysis. COPP and COPDAC appeared similarly efficacious and COPDAC was considered as a new standard consolidation treatment in HL. Half of the patients did not receive RT and there was no difference in outcome of patients treated with or without radiation therapy. Table 2 gives an overview of the Euronet protocol and the adult protocols (EORTC/LYSA/ FIL[13] and GHSG German groups) which tailored treatment according to the metabolic response (FDG-PET) after two courses of chemotherapy. Adult protocols were designed not only to reduce the indications of RT but also to select poor responding patients who might benefit from a shift to a more intensive approach.

Due to an unexpected excess of relapses occurring on the no-RT arms of EORTC/LYSA/FIL Intergroup H10 trial, the authors decided to stop enrollment in the early PET-negative part of the study and to maintain combined modality treatment as standard for early PET-negative scans.[13] These results means that RT after initial chemotherapy marginally improves the progression free survival over chemotherapy alone but at the expense of irradiating all "PET 'negative" patients; most of whom are already cured. The recently published UK study showed that in patients with stage IA-IIA HL, and PET negative after 3 cycles of ABVD, the addition of radiotherapy slightly improves their PFS rate but has no impact on their overall survival.[14] The German HD16 and HD17 are still open to accrual.

Patients with Relapsed Hodgkin Lymphoma

Patients with relapsed HL should be identified according to their prognostic factors at relapse: end-of-treatment to first progression interval <12 months versus >12 months, extranodal disease, stage and treatment given at first diagnosis (duration and intensity of chemotherapy, relapse in or outside the previously irradiated site). Presence of B-symptoms and anemia at the time of relapse proposed by the GHSG may be more accurate but represents rare events. This enables relapsing patients to be categorized in different prognostic groups.

All relapsed HL should receive second-line chemotherapy which the response could be crucial for determining the outcome. No existing randomized trial has compared the effectiveness of salvage regimens as second-line treatment for HL. These regimens have varied according to the initial chemotherapy the patient has received; for instance the given cumulative dose of anthracyclines has already reached or not. Most groups have used platinum-based regimens, such as ASHAP (doxorubicin, methylprednisolone, high-dose cytarabine, cisplatin)[15] and/or DHAP (dexamethasone, high-dose cytarabine,

Table 2: Salvage chemotherapy for Hodgkin lymphoma

Reference	Regimen	No. patients	Overall response rate
Rodriguez et al[15]	ASHAP	56	66%
Josting et al[16]	DHAP	57	80%
Brice et al[17]	IVA	43	60%
Ferme et al[18]	MINE	83	74%
Moskowitz et al[19]	ICE	65	88%
Santoro et al[20]	IGEV	91	81%
Schellong et al[21]	IEP-ABVD	176 children	85%
Linch et al[22]	Mini-BEAM	55	82%
Schmitz et al[23]	dexaBEAM	161	80%

ASHAP: doxorubicin, methylprednisolone, high-dose cytarabine, cisplatin; DHAP: dexamethasone, high-dose cytarabine, cisplatin; IVA: ifosfamide, etoposide, doxorubicin; MINE: mitoguazone, ifosfamide, vinorelbine, etoposide; ICE: ifosfamide, carboplatin, etoposide; IGEV: ifosfamide, gemcitabine, vinorelbine; IEP-ABVD: ifosfamide, etoposide and prednisone alternating with doxorubicin, bleomycin, vinblastine and dacarbazine; MiniBEAM: carmustine, etoposide, cytarabine, melphalan; dexaBEAM: dexamethasone, carmustine, etoposide, cytarabine, melphalan

cisplatin)[16] or ifosfamide/etoposide-based regimens such as IVA (ifosfamide, etoposide, doxorubicin),[17] MINE (mitoguazone, ifosfamide, vinorelbine, etoposide),[18] ICE (ifosfamide, carboplatin, etoposide),[19] IGEV (ifosfamide, gemcitabine, vinorelbine)[20] or IEP-ABVD (ifosfamide, etoposide and prednisone alternating with ABVD) for children[21] to introduce other drugs.

Intensive pre-transplant regimens, such as mini-BEAM (carmustine, etoposide, cytarabine, melphalan)[22] and dexaBEAM (dexamethasone, carmustine, etoposide, cytarabine, melphalan)[23] have severe hematological toxicity and have a 2–5% rate of toxic death.

Overall response rates (ORR) [CR and partial response (PR) >50%] of some prospective pre-transplant salvage regimens are reported in table 2; ORR ranged from 60% to 88%. RT can be used in limited cases of relapse which occur outside the initial radiation field. It can also be given after ASCT to limited fields.

High-dose chemotherapy and autologous stem-cell transplantation after standard salvage therapy is indicated for high risk patients (progression during or up to six months after first line treatment). In all other patients (early relapse or late relapse of stage IIB-III or IV), chemosensitivity to salvage conventional chemotherapy is crucial and best evaluated by FDG-PET after 2 cycles. This early evaluation decides whether this salvage chemotherapy can be continued for 2 other cycles and completed with radiotherapy (adequate response) or treatment needs to be intensified with high dose chemotherapy and autologous stem cell transplantation (inadequate or unclear response). BEAM is the most widely used conditioning regimen for relapsed HL and only little available data support the use of total body irradiation in this setting.

Role of allogeneic SCT in patients with relapsed and refractory HL remains highly controversial. The results of several studies suggested that allogenic SCT might be associated with a clinically significant graft-versus-HL effect and a lower relapse rate with respect to autologous stem cell transplant (ASCT).[24] In one study, allogeneic bone marrow transplantation was compared with autologous bone marrow transplantation using the data from the European Bone Marrow Transplantation (EBMT) registry.[25] Allografted patients (n=45) were matched to patients (n=45) who underwent ASCT based on risk factors. Allogeneic transplantation did not appear to offer any advantage when compared with ASCT in terms of progression or relapse free survival and also overall survival because of a very high transplant related mortality mainly associated with acute and chronic graft-versus-host disease and concomitant infectious episodes. The recent emphasis on reduced intensity conditioning (RIC) with allogenic SCT has gained recently interest in this procedure and could be considered an effective therapeutic approach for patients who have suffered treatment failure with a previous ASCT, those with bone marrow involvement at relapse or insufficient stem-cell collection for a second high-dose chemotherapy. Other indications should be conducted only in prospective protocols mostly regarding the role of RIC after high-dose chemotherapy and ASCT for poor-prognosis relapse as proposed by the EBMT group.[26] There is no standard treatment for patients relapsing after high-dose chemotherapy and ASCT. The decision how to treat these patients has to be made individually. New drugs are being developed in recent years that may change the management of relapsed and refractory Hodgkin lymphoma patients

Monoclonal Antibodies

SGN-35 or brentuximab vedotin is an anti CD30 antibody directed to CD30-bearing lymphomas, which include HL as well as anaplastic large cell lymphoma (ALCL). CD30 membrane glycoprotein belonging to the TNF family is not usually detected in normal tissue outside the immune system except in a small fraction of B, T or eosinophils, which makes it an ideal target for treatment with monoclonal antibodies (moAb). Anti CD30 antibody is inactive in HL but the same antibody linked to an antitubulin toxin (auristatin E) appears to

be a very active agent, the most active salvage agent ever tested in relapsed and/or refractory HL with a response rate of 75%, 34% complete remission in this population.[27] (The previously introduced new active agent for HL was gemcitabine, introduced in 2000 with less single-drug activity than brentuximab vedotin. In the initial two trials, there was a 37% response rate with 8.5% complete remissions.) Despite its exciting antitumor activity for HL, the cost of brentuximab vedotin will add considerably to the current expense of systemic therapy and the question remains that how these agents could best be combined with known active chemotherapeutic agents to improve initial response rate and durability of remission, especially in patients with advanced-stage disease. Ultimately, the question is that if this added agent results in a significant improved overall survival. The convincing evidence can only be derived from prospective randomized clinical trials that analyze precisely the additive value of the new agent. These will be essential because we seem to be entering a new era of anticancer drug based on cost and effectiveness. Safety and effectiveness of brentuximab vedotin in patients below the age of 18 has not been established.

Approximately 25% of Reed-Sternberg cells express CD20 on their cell surface. It is also postulated that the surrounding B lymphocytes which express CD20 may contribute to the survival of the Reed-Sternberg cells. Therefore, rituximab may have a role in treatment of HL by removing the supporting B cells. A pilot study of rituximab in patients with recurrent, classic HL, regardless of whether Reed-Sternberg cells expressed CD20, was conducted.[28] In total, 22 patients with nodular sclerosis histology were evaluable for treatment response. Five patients (22%) achieved remission (one CR and four PR). A phase 2 trial of the German Hodgkin Lymphoma Study Group evaluated the safety and efficacy of rituximab in patients with relapsed lymphocyte-predominant Hodgkin lymphoma or other CD20 (+) subtypes of Hodgkin disease (HD). The overall response in 14 assessable patients was 86%, with 8 complete remissions and 4 partial remissions, and 2 patients with progressive disease.[29]

Yttrium-90 radiolabeled humanized monoclonal antibody to CD25 (^{90}Y-labeled daclizumab) has been evaluated in 46 patients with relapsed and refractory HL. There were 9 CR and 14 PRs. Interestingly, many of the responses were seen in patients with CD25-negative malignant cells but with surrounding CD25-positive T cells within the tumor microenvironment.[30]

Other Novel Agents

HRS cells aberrantly express a variety of pro-survival proteins, such as nuclear factor- kappaB (NF-kappaB), Jak/STATs, Akt/mTOR, Notch-1 and extracellular signal-regulated kinase (ERK), that can be targeted by small molecules. These proteins can be targeted either by selective small-molecule inhibitors such as Jak-2, mTOR and B-cell lymphoma (Bcl)-2 family inhibitors or by broad inhibitors that modulate several unrelated molecules, such as histone deacetylase (HDAC), proteasome inhibitors and heat shock protein 90 (HSP90) inhibitors.

Several of the agents belonging to the histone deacetylase inhibitors are being employed in early clinical trials in relapsing HL. They have an antiproliferative effect on Hodgkin cells and possibly an immune-mediated effect. Panobinostat, is one of those. In contrast to brentuximab vendotin, panobinostat is an oral drug that is administered three times a week in 21-day cycles. In a phase II study, there was notable activity with a response rate of 21.7%; however, there was some reduction in tumor size in 74% of patients. Nevertheless, its high myelotoxicity would certainly limit the ability to combine panobinostat with other myelotoxic drugs.[31]

Lenalidomide, a derivate of thalidomide is thought to be an immunomodulating agent. Its mechanism of action is to interact with the microenvironment of the tumor; it has anti-angiogenic and immunomodulating properties and induces directly the death of malignant B cells. The microenvironment definitely plays variable beneficial roles in HL survival with production of cytokines, interleukins and TGFβ and existence of regulatory T-lymphocytes. Lenalidomide has been evaluated in patients with relapsed and refractory HL. In the first study, 15 patients were treated of whom12 patients were evaluated; there were one CR and three PRs.[32] In another study; among 14 evaluable patients, two PR were observed with no CR.[33]

Conclusion

Despite the high curability rate of HL, there is still a huge space for improving the treatment of refractory or relapsed patients. There are a list of drugs in clinical trials demonstrating benefit against HL, but it is certainly too soon to know whether all these drugs will contribute to improving survival more than the customary treatments we use for these patients. We also need to go deeper into the understanding and handling of toxicity and interactions with other drugs and, finally, how to rationalize their use and make this sustainable in the current economic situation.

Conflict of Interest: None declared.

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IJBC 2015; 7(5): 213-219

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

ORIGINAL ARTICLE

Methylation Status of SOX17 and RUNX3 Genes in Acute Leukemia

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ARTICLE INFO

Article History:
Received: 01.06.2015
Accepted: 13.09.2015

Keywords:
Acute myeloid leukemia
Acute lymphoblastic leukemia
RUNX3
SOX17
DNA Methylation
Prognosis

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ABSTRACT

Background: Several studies have examined the presence of DNA methylation of CpG islands in leukemia. Methylation of SOX17 and RUNX3 genes may play a role in leukemogenesis through silencing tumor suppressor genes. We investigated the methylation status of SOX17 and RUNX3 genes in patients with acute leukemia.

Methods: In this case-control study, peripheral blood samples from 100 AML and 100 ALL patients and 100 healthy controls were collected. Isolated DNA was treated with sodium bisulfite and methylation status was examined by methylation specific PCR (MS-PCR) with primers specific for methylated and unmethylated sequences of SOX17 and RUNX3 genes.

Results: The frequency of hypermethylation of SOX17 and RUNX3 genes were 36% and 28%I in patients with acute myeloid leukemia (AML), and 21% and 22% in patients with acute lymphoblastic leukemia (ALL), respectively. Aberrant methylation of these genes was found in all FAB classifications of AML and ALL. Hypermethylation of SOX17 (P=0.055) and RUNX3 (P=0.003) genes were associated with FAB-M0 and M1 subtypes of AML, respectively. Also, aberrant methylation of RUNX3 gene was associated with FAB-L1 subtype of ALL (P=0.053). There was not any significant association between hypermethylation of SOX17 and RUNX3 genes and clinical parameters of patients with leukemia including sex, age, WBC, and platelet counts.

Conclusion: Hypermethylation of SOX17 and RUNX3 genes was seen in patients with acute leukemia. Moreover, no significant association was observed between hypermethylation of SOX17 and RUNX3 and induction of remission.

Please cite this article as: Ghasemi A, Ghotaslou A, Ghaffari K, Mohammadi M. Methylation Status of SOX17 and RUNX3 Genes in Acute Leukemia. IJBC 2015; 7(5): 213-219.

Introduction

DNA hypermethylation of promoter-associated CpG islands of tumor suppressor and DNA repair genes has been the most studied epigenetic alteration in human neoplasia.[1] In acute lymphoblastic leukemia (ALL), promoter hypermethylation is reported to be associated with a poor prognosis.[2] In acute myeloid leukemia (AML), many tumor suppressor genes are silenced through DNA methylation such as CDKN2B, P73 and suppressor of cytokine signaling.[3] Epigenetic disorders, in contrast to genetic changes are reversible and the role of DNA demethylating agents such as azacitidine and 5-azadeoxycytidine (decitabine) has been established in the treatment of hematopoietic malignancies.[4-7] DNA methylation patterns are often altered in cancer cells with increased level of the DNA methyltransferase.[8-10] Moreover, widespread genomic hypomethylation and simultaneous regional increase in DNA methylation patterns have been reported.[11] Molecular genetic alterations affecting NPM1 (nucleophosmin1) and FLT3

genes as well as WT1 (Wilms' Tumor) are among known important prognostic factors in AML.

In recent years, epigenetic disorders including methylation of tumor suppressor genes such as Wnt (Wingless and integration site) gene and its antagonist, Dickkopf-1 (DKK-1) has been shown to play some role in AML pathogenesis.[12] These alterations may lead to differentiation and apoptosis arrest in leukemic blasts as well as increase in proliferation and self-renewal.[13] Epigenetic aberrations in Wnt pathway are critical for the initiation of a variety of epithelial cancers and it has been demonstrated that abnormalities of this pathway are also common in hematopoietic malignancies.[14-16] In normal cells, Wnt signaling and β-catenin localization are tightly controlled by a number of intracellularly secreted inhibitory proteins including Dickkopf 1, 2 (DKK1,2), serine/ threonine kinase 11 (LKB1), Ras association domain-containing protein 1, runt-related transcription factor 3 (RUNX3), secreted frizzled related proteins 1, 2, 4, 5 (sFRP1, 2, 4, 5), SRY-box containing gene 17 (SOX17), and WNT inhibitory factor 1 (WIF1).[14,17] In some malignancies like colorectal cancers, head and neck tumors and gastric cancers, aberrant Wnt signaling pathway has been shown to cause uncontrolled cell proliferation.[18] β-catenin is an intracellular regulator of transcription that is associated mainly with epithelial cancers. Wnt controls the cytoplasmic level and stability of β-catenin.[19]

In the absence of Wnt ligand and its protective role, β-catenin level decreases due to destruction by Casein Kinase 1 and Glycogen Synthase Kinase 3b enzymes.[20] When the ligand adheres to its receptor (frizzled receptor), activates Dvl (disheveled) proteins.[21] Having accumulated in cytoplasm, β-catenin migrates to nucleus where it causes expression of some genes involved in cell proliferation and differentiation.[18]

It has recently been demonstrated that both chromosomal alterations and FLT-3 mutations associated with AML pathogenesis, affect the Wnt signaling pathway.[22] SRY-related (Sox) transcription factors contain a HMG DNA-binding domain that regulate stem cell identity and function in multiple tissues.[23] Sox17 activates endodermal target genes and is required for the formation of endoderm and vascular endothelium.[24-26] Sox17 also plays an important role in the maintenance of fetal and neonatal hematopoietic stem cells.[27] Reduction of mature blood cell formation in zebrafish by RUNX3 depletion suggested a role for RUNX3 in hematopoiesis.[28] The role of RUNX3 in tumorigenesis and its potential involvement in hematopoiesis suggests a role for this transcription factor in hematological malignancies. However, genetic alterations of RUNX3 have not been reported in acute myeloid leukemia.[29]

Methylation of SOX17 and RUNX3 genes leads to loss of their inhibitory effect on Wnt pathway. Then cytoplasmic and nuclear levels of β-catenin enhances that as a transcription factor makes some genes associated in cell cycle regulation like MYC, COX and Cyclin D to be expressed.[30] we aimed to investigate the methylation status of SOX17 and RUNX3 genes in de novo non-M3 patients with AML and ALL at diagnosis.

Patients and Methods

One hundred patients with non-M3 AML and 100 patients with ALL and also 100 healthy controls were enrolled. At the beginning of the study, informed consent was obtained from all groups.

All patients were divided in FAB classification groups. The clinical parameters consist of white blood cell count, platelet, age, hemoglobin, and rate of recovery following induction chemotherapy extracted from patients medical records. Mononuclear cells of drawn samples including leukemic blast cells were isolated by concentration gradient sedimentation using Ficoll-hypaque followed by DNA extraction by saturated salt standard method.[17] In the next step extracted DNA underwent bisulfite conversion with the Epitect Bisulfite kit (Qiagen, Germani, Inc cat no. 59695) using the maunfacturer's instructions. By this treatment unmethylated cytosine converted to uracil where methylated cytosine stayed intact. Then the methylation status of SOX17 and RUNX3 genes was investigated using MSP (Methylation specific PCR) technique. MSP is a type of PCR used to investigate the methylation of CpG islands. In this method we used 2 pairs of primers specified for checking the methylated or unmethylated residue. These primers are shown in table 1, accompanied by product values.

Four MSP reactions using methylated and unmethylated primers related to SOX17 and RUNX3 were administered for each patient. In methylation testing we used 2 µl of DNA previously treated with Bisulfite, 4 µl of dH20, 12 µl of Master mix, 0.5 µl of forward primer and 0.5 µl of reverse primer while in order to investigate the unmethylated status. We used 2 µl of DNA, 7.5 µl of

Table1: SOX17 and RUNX3 gene primers sequence, annealing temperature and product size for MSP assays

Primer	Sequence (5' to 3')	Annealing temperature	Product Size (bp)
SOX17- MF	CAAAAACGAATCCCGTATCCGACG	62	79
SOX17- MR	TTGCGTTAGTCGTTTGCGTTC		
SOX17-UF	CAAACCAAAAACAAATCCCATATCCAACA	60	91
SOX17- UR	GATTTTGTTGTGTTAGTTGTTTGTGTTTG		
RUNX3-MF	GGCGGTCGTCGGGTTAGCGAGGTTTC	62	87
RUNX3- MR	CCCGAACCTCAAAACGCAAAAAACGACG		
RUNX3-UF	GTGGGTGGTTGTTGGGTTAGTGAGGTTTT	60	92
RUNX3-UR	AACCCAAACCTCAAAACACAAAAAACAACA		

M: Methylated, U: Unmethylated, F: Forward, R: Reverse

Figure 2: Karyotype study of the patient showed 45, XY, -7 (monosomy 7)

dH20, 12 µl of master mix, 0.5 µl of forward primer, 0.5 µl of reverse primer and 0.5 µl of MgCl2. In the first step of MSP, reaction components put in pre-thermal conditions including 99ºC for 1 minute and 95ºC for 3 minutes followed by 35 cycles including 99ºC for 10 seconds, 95ºC for 30 seconds, 60ºC for 30 seconds (SOX17 and RUNX3-UM Primer), 62ºC for 30 seconds (SOX17 and RUNX3-M Primer) and 70ºC for 5 minutes (extension). In this study, we used EpiTect PCR control DNA kit (Qiagen Inc cat no. 59695) containing unmethylated and completely methylated DNAs as negative and positive controls, respectively. Electrophoresis on 4.5% agarose gel was done in order to identify MSP products (figures 1 and 2). Fisher's exact two-sided tests, Mann-Whitney U test were used as approapriated. Data were analyzed using SPSS software, version 21(version 21, SPSS Inc Chicago, IL). P-value less than 0.05 were considered significant.

Results

The AML group included 70 (70%) men and 30 (30%) women and the ALL group included 60 (60 %) men and 40 (40 %) women, respectively. Mean±SD age of patients with AML and ALL was 43.5±10 years (range: 15-75 years) and 43.5±10 years (range: 13-62 years), respectively. WBC and platelet counts in patients with AML were 0.45-375×10^9 /L and 0.015-280×10^9 /L (mean values were 15.2±0.16×10^9 /L and 95±0.65×10^9 /L), respectively and in ALL patients WBC and platelet counts were 0.320-150×10^9 /L and 30-320×10^9 /L, respectively (mean values were 11±0.120 and 80±0.330×10^9 /L), respectively.

SOX17 gene found to be hemi-methylated in 30 (30%) patients with AML and 32 (32%) patients with ALL, completely methylated in 36 (36%) patients with AML and 21(21%) patients with ALL and completely unmethylated in 34 (34%) patients with AML and 47 (47%)patients with ALL , while RUNX3 gene was hemi-methylated in 42 (42%) patients with AML and 46 (46%) patients with ALL, completely methylated in 28 (28%) patients with AML and 22 (22%) patients with ALL and completely unmethylated in 30 (30%) patients with AML and 32 (32%)patients with ALL. Methylation in SOX17 and RUNX3 genes was not seen in the control group. Correlation between hypermethyaltion of SOX17 and RUNX3 genes and clinical and laboratory features of leukemia patients are shown in tables 2 and 3, respectively. In patients with AML, frequency of hypermethylation of SOX17 and RUNX3 genes were 36% and 28% and in patients with ALL, it was 21% and 22%, respectively.

Patients with AML with hypermethylation of RUNX3 genes had higher hemoglobin than those without hypermethylation (P=0.065). Aberrant methylation of these genes was found in all FAB classifications of AML and ALL. Hypermethylation of SOX17 (P=0.055) and RUNX3 (P=0.003) genes were associated with FAB-M0 and -M1 subtype of AML, respectively (table 2). Also, aberrant methylation of RUNX3 gene was associated with

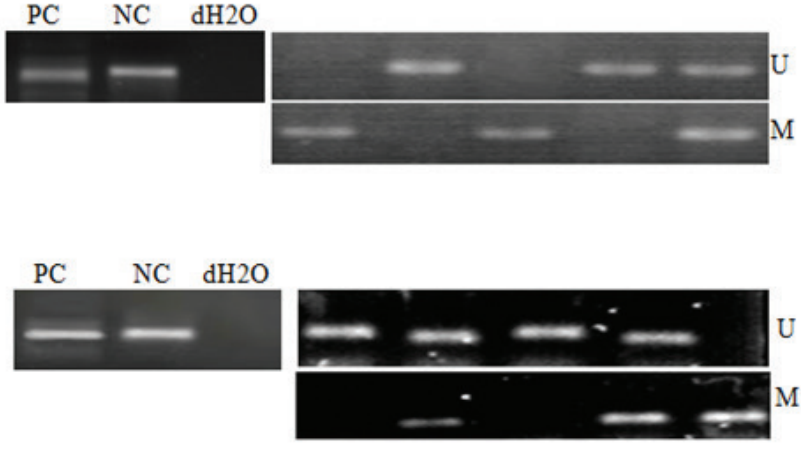

Figure 1: MSP analysis of SOX17 and RUNX3 genes in AML patients and normal control. PC: Positive control; NC: Negative control; P: Patient; M: Methylated; U: Unmethylated. dH2O served as a blank control.

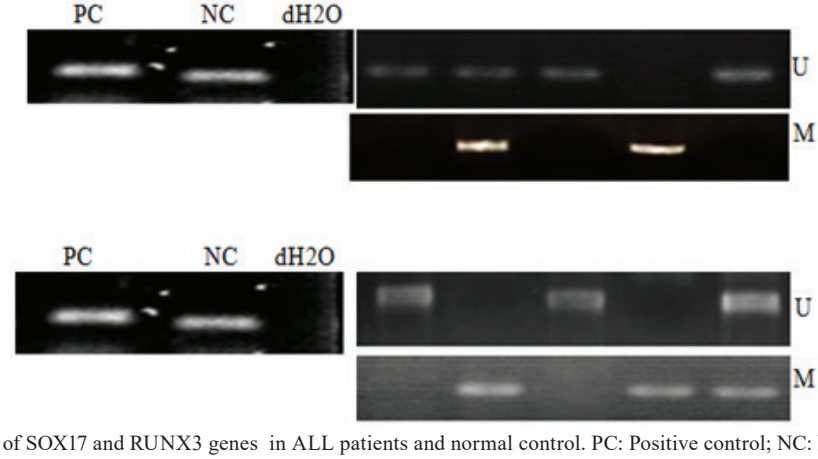

Figure 2: MSP analysis of SOX17 and RUNX3 genes in ALL patients and normal control. PC: Positive control; NC: Negative control; P: Patient; M: Methylated; U: Unmethylated. dH2O served as a blank control.

Table 2: Correlation between hypermethylation of SOX17 and RUNX3 genes and laboratory and clinical symptoms of AML patients.

	SOX17			RUNX3		
Characteristics	**M**	**U**	**P**	**M**	**U**	**P**
Number of Patients, (%)	36 (36)	64 (64)		28 (28)	72 (72)	
Age, median (range) years	39.6±3 (21-65)	35.1±6 (18-62)	0.415	42±5 (23-70)	48±8 (15-72)	0.432
Sex, %			0.175			0.629
Male	22	48		21	49	
Female	14	16		7	23	
WBC count (10^9/L, median)	15.2±3	25.4±7	0.311	34.1±5	30.2±2	0.265
Platelet count (10^9/L, median)	120.6±10	212±8	0.524	98±4	135±6	0.321
Hb, g/dL (median)	8.6±0.8	9.1±0.5	0.201	8.9±0.2	8.3±0.6	0.065
FAB type, n (%)						
M0	4 (11.1)	1 (2.7)	0.055	3 (10.7)	2 (2.7)	0.132
M1	6 (16.6)	8 (12.5)	0.563	9 (32.1)	5 (6.9)	0.003
M2	10 (27.7)	21 (32.8)	0.658	6 (21.4)	25 (34.7)	0.235
M4	8 (22.2)	16 (25)	0.812	6 (21.4)	18 (25)	0.798
M5	6 (16.6)	14 (21.8)	0.610	3 (10.7)	17 (23.6)	0.175
M6	2 (5.5)	4 (6.2)	0.999	1 (3.5)	5 (6.9)	0.999
Outcome, n (%)						
Complete remission	22 (61.1)	45 (70.3)	0.381	18 (64.2)	49 (68)	0.814
Death	4 (11.1)	7 (10.9)	0.999	3 (10.7)	8 (11.1)	0.999
Relapse	10 (27.7)	12 (18.75)	0.322	7 (25)	15 (20.8)	0.788

AML: Acute Myeloblastic leukemia, Hb: Hemoglobin, WBC: White blood cell, FAB: French-American-British, M: Methylated, U: Unmethylated

Table 3: Correlation between hypermethylation of SOX17 and RUNX3 genes and laboratory and clinical symptoms of ALL patients.

	SOX17			RUNX3		
Characteristics	**M**	**U**	**P**	**M**	**U**	**P**
Number of Patients, (%)	21 (21)	79 (79)		22 (22)	78 (78)	
Age, median (range) years	27.6±5 (19-55)	30±3 (13-62)	0.325	22±2 (17-55)	25±6(15-60)	0.183
Sex, %			0.317			0.999
Male	15	45		13	47	
Female	6	34		9	31	
WBC count (10^9/L, median)	9.5±2	15±2.5	0.632	14.3±2	18±3	0.331
Platelet count (10^9/L, median)	110.5±8	200±5	0.424	89±6	125±4	0.543
Hb g/dL(median)	8.9±0.6	10.5±1	0.187	9.2±0.2	9.8±0.8	0.139
FAB type, n (%)						
L1	4 (19)	12 (15.1)	0.739	6 (27.7)	10 (12.8)	0.053
L2	9 (42.8)	38 (48.1)	0.807	8 (36.3)	39 (50)	0.335
L3	8 (38)	29 (36.7)	0.999	8 (36.3)	29 (37.2)	0.999
Outcome, n (%)						
Complete remission	15 (71.4)	68 (86)	0.187	17 (77.2)	66 (84.6)	0.520
Death	2 (9.5)	3 (3.8)	0.282	2 (9.1)	3 (3.8)	0.303
Relapse	4 (19.1)	8 (10.2)	0.271	3 (13.7)	9 (11.5)	0.723

ALL: Acute Lymphoblastic leukemia, Hb: Hemoglobin, WBC: White blood cell, FAB: French-American-British, M: Methylated, U: Unmethylated

FAB-L1 subtype of ALL (P=0.053, table 3).

There was no significant association between hypermethylation of SOX17 and RUNX3 genes and clinical parameters of patients with leukemia including sex, age, WBC, and platelet counts (tables 2 and 3).

Twenty two out of 100 patients with AML developed relapse in whom 10 and 12 patients were hypermethylated for SOX17 and RUNX3 genes, respectively. There was no significant association between hypermehylation of both SOX17 and RUNX3 genes and relapse of patients with AML (P=0.322 and P=0.788, respectively). 67 (67%) patients with AML developed complete remission after induction chemotherapy; of whom 22 and 18 were hypermethylated for SOX17 and RUNX3 genes (P=0.381 and P=0.814, respectively). There was no significant association between hypermethylation

in the SOX17 and RUNX3 genes and achievement of induction remission in patients with AML (table 2). Twelve out of 100 patients with ALL developed relapse whom 4 patients were hypermethylated for SOX17 and 3 for RUNX3 genes. There was no significant association between hypermehylation of both SOX17 and RUNX3 genes and relapse of patients with ALL (P=0.271 and P=0.723, respectively).

Demographic and clinical features of 93 patients with ALL were available of whom 83 (93%) patients achieved complete remission of induction; 15 and 17 were hypermethylated in the SOX17 and RUNX3 genes, respectively (P=0.187 and P=0.520, respectively). There was no significant association between hypermethylation in the SOX17 and RUNX3 genes and remission rate in patients with ALL (table 3).

Discussion

In this study we investigated the methylation status of SOX17 and RUNX3 genes in newly diagnosed patients with AML and ALL. The results of this study showed that hypermethylation of SOX17 and RUNX3 genes occurred with a frequency of 36% and 28% in patients with AML and 21% and 22% in patients with ALL, respectively. Understanding the roles of Wnt/β-catenin signaling in survival, proliferation and differentiation of hematopoietic stem cells resulted in developing the hypothesis that this signaling pathway may be involved in leukemogenesis.[31-33]

More recently, inactivation of RUNX3 was reported in a wide range of other cancer types.[34] There is evidence that RUNX3 is inactivated by gene silencing or protein mislocalization in more than 80% of gastric cancers.[35,36]

Frequent SOX17 gene methylation has been detected in colon, liver, and breast cancers.[37-39] SOX17 belongs to the high-mobility group (HMG)-box transcription factor superfamily, which is homologous to the sex-determining gene SRY.[40] SOX17 has been reported to promote degradation of β-catenin/TCF via a GSK3β-independent mechanism in Wnt signaling pathway and has been recognized as an important antagonist and inhibitor of the canonical Wnt signaling pathway.[41,42] Hypermethylation of other inhibitors of Wnt signaling pathway has been found in some malignancies such as SFRP, WIF1 and DKK-1 gene methylation in AML.[43,44] Yu and colleagues demonstrated that promoter methylation of the Wnt/β-Catenin signaling antagonist DKK-1 is associated with poor survival in gastric cancer.[45]

The percentage of patients with AML with aberrant methylation was 66% and 70% for SOX17 and RUNX3 and in patients with ALL, 53% for SOX17 and 68 % for RUNX3. The frequency of hypermethylation of SOX17 and RUNX3 in patients with AML in this study was higher than those reported by Griffiths and co-workers (29% and 27 %, respectively; total: 56%).[44] These probably reflect the difference in patient selection and ethnic diversity. SOX17 and RUNX3 genes are epigenetic targets in AML patients which are inactivated through methylation processes.[44,46]

Interestingly, methylation-associated RUNX3 silencing was detected in half of the ALL and CML cell lines, suggesting that RUNX3 methylation occurs in certain types of hematological malignancies.[46] Moreover, Cheng and colleagues pointed out that unlike in AML, RUNX3 was epigenetically silenced by promoter methylation in t(12;21)-positive cells. Whether RUNX3 is also transcriptionally repressed by TEL-RUNX1 awaits further investigation.[46]

Our results showed that aberrant methylation of SOX17 and RUNX3 occurred in all FAB-AML and -ALL subtypes. Patients with FAB-M0 and -M1 subtype had the highest incidence of hypermethylation of SOX17 (80 %, P=0.055) and RUNX3 (65 %, P=0.003), respectively; whereas those with M6 subtype had the lowest incidence of SOX17 (33.4 %, P=0.999) and RUNX3 (16. %, P=0.999) hypermethylation, respectively. Likewise, patients with FAB-L1 subtype of ALL had the highest incidence of hypermethylation of SOX17 (25%, P=0.739) and RUNX3 (37.5 %, P=0.053), respectively; whereas those with L2 subtype had the lowest incidence of SOX17 (20%, P=0.8) and RUNX3 (17%, P=0.3), respectively. In this study, we did not observe any significant association between hypermethylation of these genes and prognostic factors.

Griffiths and co-workers reported that methylation of SOX17 was associated with a trend toward increased risk of relapse and methylation of sFRP4 was associated with an increased risk for death.[44] In our study, induction of remission was observed in 67% and 83% in patients with AML and ALL, respectively. In our study, no significant association was observed between hypermethylation of SOX17 and RUNX3 and induction of remission.

Conclusion

We found that CpG island methylation of SOX17 and RUNX3 genes is a common event in patients with AML and ALL. Patients with FAB-M0 and -M1 subtype and FAB-L1 subtype of ALL had the highest incidence of hypermethylation of SOX17 and RUNX3. Moreover, no significant association was observed between hypermethylation of SOX17 and RUNX3 and induction of remission.

Conflict of Interest: None declared.

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IJBC 2015; 7(5): 220-222

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

ORIGINAL ARTICLE

Correlation Between Demographic and Laboratory Variables in Adult Patients with Acute Idiopathic Thrombocytopenic Purpura in West Iran

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ARTICLE INFO

Article History:
Received: 10.06.2015
Accepted: 20.09.2015

Keywords:
Idiopathic thrombocytopenic purpura
Helicobacter pylori infection
Acute
Chronic
Hemoglobin
WBC count

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ABSTRACT

Background: Idiopathic thrombocytopenic purpura is an autoimmune blood disorder in which platelet destruction is mediated by anti-platelet antibodies. There are two forms of ITP: acute and chronic. The aim of the present study was to evaluate the clinical variables in adult patients with acute ITP in west of Iran.
Patients and Methods: Medical records of adult patients with diagnosis of acute ITP referring to Hematology Clinic of Kermanshah from year 2004-2014 were analyzed. Demographic and hematologic data and status of *H pylori* infection of the patients were extracted.
Results: There were records of fifty-three patients diagnosed with acute ITP. Mean age at diagnosis was 39.1 years (±13.3) ranging from 14-68 years. Twenty patients (37.7%) were male. Out of 53 patients, 25 cases (47.2%) were positive for *H pylori* infection. There was significant association between Hb and platelet with sex of the patients (P≤0.05).
Conclusions: Mean age of adult patients with acute ITP was more than figures expected in chronic ITP patients. In addition, Prevalence of *H pylori* infection in acute ITP patients was more than chronic ITP patients.

Please cite this article as: Payandeh M, Sadeghi M, Sadeghi E, Hosseini M. Correlation Between Demographic and Laboratory Variables in Adult Patients with Acute Idiopathic Thrombocytopenic Purpura in West Iran. IJBC 2015; 7(5): 220-222.

Introduction

Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder in which platelet destruction is mediated by anti-platelet antibodies. There are two forms of ITP: acute and chronic.[1] The acute form is frequently observed among children, but the chronic form mainly inflicts adults. There are numerous differences and similarities in clinical and laboratory findings between children and adult patients with ITP.[2] Helicobacter pylori (H. pylori) is a gram-negative, spiral shaped bacterium that colonizes the gastric mucosa. It is a major cause of gastritis and peptic ulcer disease as well as the development of gastric malignancies.[3] In several studies, an association between H. pylori infection and a number of autoimmune disorders such as adult ITP has been proven.[4-6] Helicobacter pylori is highly prevalent in developing countries and is common among 57–91% of the Iranian population[7]. We aimed to evaluate the clinical variables and status of H. pylori infection in adult patients with acute ITP in west of Iran.

Patients and Methods

During 2004-2014, fifty-three patients with acute ITP (platelet range=50-99 x10^3/μL) referred to Hematology Clinic in Kermanshah, west Iran. Age, sex, Hb, WBC count, platelet count were analyzed. All patients had also been screened for H. pylori infection using H. pylori urea breath test (UBT) and serum H. pylori antibody.

Correlation between Hb, WBC and platelet counts with H pylori infection and age of the patients was assessed

using t test. The association between H. pylori infection and sex was assessed by Chi-square test (Fisher's exact rest). P<0.05 was considered statistically significant. Data were analyzed using SPSS software, version 19.

Results

The mean±SD age at diagnosis was 39.1±13.3 years (range=14-68 years). 25 (47.2%) patients were less than 40 years of age. Twenty (37.7%) patients were men and 33 (62.3%) patients were women. Out of 53 patients, 25 (47.2%) had H. pylori infection (table 1).

Mean platelet count at diagnosis was $71\times10^3/\mu L$ (range=52-99), mean Hb was 13.5 g/dL (range=11-18) and mean WBC was $8\times10^3/\mu L$ (range=2.2-40).

We compared age, WBC, platelet count and H. pylori infection in patients with acute ITP with respect to sex. There was only a significant association between platelet count and sex (P≤0.05, table 2).

Table 3 shows the association between age, Hb, WBC and platelet count with H. pylori infection. No association was found between these variables and H. pylori Infection (P>0.05).

Discussion

ITP is an acquired autoimmune disorder characterized by thrombocytopenia and mucocutaneous bleeding.[8] It is commonly assumed that ITP results from autoantibodies causing accelerated platelet destruction. Recent data suggests that autoantibodies may also inhibit platelet production.[9] Diagnosis of ITP is complex and is based on exclusion of other causes of thrombocytopenia.[10]

A study on patients with chronic ITP from Iran showed that 66 out of 129 (51.2%) patients with a mean±SD age of 29.2±7.0 years (range=18-46 years), were female.[11] Elezović et al.[12] reported that 136 out of 167 patients with chronic ITP were women (81.4%) and median age of their patients was 35 years (range=17-74 years). In another study[1] on 90 patients with chronic ITP, mean±SD age at diagnosis was 36.7±14.2 years (range, 14- 69 years) and 77.8% were women. In our study on patients with acute ITP, the mean±SD age of the patients was 39.1±13.3 years (range=14-68 years) and 62.3% were women, which is almost similar to the other studies. Frederiksen and colleagues found a mean age of 56 years in 221 patients with acute ITP.[13] Another study reported that there was no significant correlation between age or platelet count with H. pylori infection which the results were in accordance with our study.[14]

The prevalence of H. pylori infection in adult patients with ITP has been systematically reviewed which was found not to be different from that reported in the general population when it was matched for age and geographical

Table 1: The Basic characteristics of all of patients with acute ITP (n=53)

Variables	n (%)	Mean±SD	Range
Age(year)		39.1±13.3	14-68
<40	25 (47.2)		
≥40	28 (52.8)		
Sex			
Male	20 (37.7)		
Female	33 (62.3)		
H. pylori Infection			
Positive	25 (47.2)		
Negative	28 (52.8)		

Table 2: The variables in acute ITP patients based on sex (n=53)

Variables	Sex (Mean±SD)		P value
	Male	Female	
Age (year)	42.7±13.5	36.8±12.9	P=0.1*
White Blood Cell ($x10^3/\mu L$)	9.2±7	7.7±2.4	P=0.3*
Platelet ($x10^3/\mu L$)	76±13	69±11	P=0.05*
H. pylori Infection, n (%)			
Positive	12 (60)	13 (39.4)	P=0.1**
Negative	8 (40)	20 (60.6)	

*T-test, **Chi-Square Test (Fisher's Exact Test)

Table 3: Variables in acute ITP patients in terms of *H pylori* Infection (n=53)

Variables	*H pylori* Infection(mean±SD)		P value
	+	-	
Age (year)	37.6±14.4	40.3±12.4	P=0.4*
Hemoglobin (g/dL)	13.9±1.6	13.2±1.4	P=0.08*
White Blood Cell ($x10^3/\mu L$)	9±6.9	7.6±2.4	P=0.3*
Latelet ($x10^3/\mu L$)	73±14	70±11	P=0.3*

*T-test

area.[15] In Japan, the prevalence of H. pylori infection is greater than 70%. A prevalence of 22% for H. pylori infection has been reported in North American patients with chronic ITP.[16] This prevalence has been reported about 29% in adult patients with ITP of white French origin.[16] A study on patients with chronic ITP[1] showed a prevalence of 27.8% for H. pylori infection. In our study, the prevalence of H. pylori infection in acute ITP patients was 47.2% which was greater than other studies except from Japan. Therefore, we can assume that prevalence of H. pylori infection in acute ITP patients was more than what is expected from other studies on patients with chronic ITP.

A case of ITP associated with splenic tuberculosis has been reported that hemoglobin and WBC count were 12g/dl and 8×10^3/µL, respectively.[17] Another study on 93 patients with chronic ITP showed Hb measurements in range of 9.6-17.5 g/dL and WBC counts 3.9-20.5($\times10^3$/µL), respectively[2]. In our study in acute ITP patients, mean of Hb and WBC counts were 13.5g/dl and 8×10^3/µL, respectively. These results showed that probably there has been no correlation between Hb and ITP in terms of chronicity (acute or chronic). In our study which analyzed adult patients with acute ITP, mean platelet counts was significantly higher in men than women ($P<0.05$).

Conclusion

Mean age for adults with acute ITP was more than what is expected among patients with chronic ITP. Moreover, it can be assumed that the prevalence of H. pylori infection in patients with acute ITP is more than those with chronic ITP.

Conflict of Interest: None declared.

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IJBC 2015; 7(5): 223-226

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

ORIGINAL ARTICLE

The Prognostic Value of White Blood Cells Count in Patients with Myocardial Infarction

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ARTICLE INFO

Article History:
Received: 27.06.2015
Accepted: 1.09.2015

Keywords:
Leukocytosis
Mortality Rate
Myocardial infarction
WBC Count
Prognostic factor

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ABSTRACT

Background: Ischemic heart disease and acute myocardial infarction is one of the most dramatic manifestations in one of the most investigated fields in the past few decades. In this study, the prognostic value of white blood cells count in patients with myocardial infarction (MI) was investigated in a six months follow-up.
Methods: In this cohort study, 106 patients with MI were investigated. White blood cell counts were assessed 48 hours after MI and the location of MI was determined using ECG. Mortality rate was determined and their correlation with leukocytosis was analyzed up to 6 months of follow-up. Binary logistic regression analysis was applied between factors such as mortality rate, location of the myocardial infarction, sex, hemoglobin and WBC count.
Results: Mean age of the patients was 62.5±13.3 years. 76.4% were men. 26% of patients had leukocytosis. Leukocytosis was significantly correlated with mortality in a six-month follow-up period (P<0.001). Fifteen (14.2%) patients died during the first three months of follow-up, of which 13 (86.7%) had leukocytosis. It was also shown that mean age of the patients and anemia in deceased group were significantly more than the survived group.
Conclusion: High WBC count in the first 48-h after MI can be regarded as a poor prognostic factor and it has an independent role in determining prognosis of patients with MI for the next six months.

Please cite this article as: Shahriari Ahmadi A, Amirfarhangi A, Gheisoori A, Arabi M, Mahmoudian AR, Payandeh M, Sadeghi M. The Prognostic Value of White Blood Cells Count in Patients with Myocardial Infarction. IJBC 2015; 7(5): 223-226.

Introduction

Coronary artery disease is the major cause of death in most modern societies across the globe. In addition, the disease leads to high morbidity, disability and loss of productivity. The clinical symptoms of coronary heart disease contain a spectrum of silent ischemia to chronic stable angina, unstable angina, acute myocardial infarction, ischemic cardiomyopathy, sudden cardiac death, arrhythmias and cardiogenic shock. Currently, 900,000 people are diagnosed with acute myocardial infarction (AMI) in the United States each year, of those about 225,000 die due to arrhythmia or heart failure.[1-5]

Systemic inflammation is triggered by myocardial infarction which is associated with the release of hematopoietic precursor cells from bone marrow into blood stream.[6,7] Understanding the cellular changes during AMI could be practically prognostic.[8] Likewise, it has been shown that immunologic changes following AMI have prognostic value for severity of AMI; however, are independent of risk factors and number of arteries involved.[9] It has been show that there is a significant correlation between the ischemic severity and the magnitude of cellular changes[10] as a consequence of acute phase response.[11]

Based on previous reports on importance of the cellular changes following AMI, this study was aimed to investigate prognostic value of the changes in hemoglobin and leukocytes in patients with myocardial during a six-month period in Iranian patients.

Patients and Methods

In a cohort study, 106 patients admitted in CCU ward of Rasul-Akram Hospital, Tehran, Iran were enrolled onto this study. Diagnostic criteria of AMI were typical or atypical chest pain that were confirmed by changes in pattern of ECG and increased levels of blood or cardiac enzymes. Patients with a history of blood disorders, including chronic anemia, leukemia and lymphoma were excluded. Complete blood count for each patient was performed within 48 hours after admission and monthly during a 6-month follow-up period after discharge. Several parameters including age, sex, mortality rate, location of myocardial infarction, and changes in leukocyte and hemoglobin levels were studied.

Data were analyzed using SPSS version 18 statistical software. The frequency for qualitative variables and mean and standard deviation for quantitative variables were calculated. Chi-square and Fisher's exact tests were used for hypothesis analysis. Binary logistic regression model was used to identify the prognosis factors during the 6-month period. The confidence limit was 95% and P<0.05 was statistically significant.

Results

In the current study, demographic and paraclinical data of 106 patients with AMI were investigated. 81 (76.4%) out of 106 patients were male. Mean age of the patients was 62.5±13.3 years (ranged from 40 to 89 years). Patients were divided into two age groups of ≤60 years (54 patients or 50.9%) and >60 years (52 patients or 49.1%). Table 1 shows the demographical parameters.

Average WBC

Mean WBC count of patients was 8616±2971/µl (ranged between 4800-22,000/µl). It was found that 28 patients (26.4%) had a WBC count above 10,000/µl, an indication of leukocytosis. Mean hemoglobin level of patients was 13.2±1.7 g/dl (range: 8.6-16.9 g/dl). Mean number of platelets were 205000±61000 /µl, which ranged from 99,000 to 408000.

Frequency of Death

13 out of fifteen patients who died during a period of six-month follow-up had leukocytosis (86.7%). A statistically significant correlation was found between mortality rate and leukocytosis (table 1). It was shown that mean age of the patients and incidence of anemia in deceased group was significantly more than survived group (P<0.001 and P=0.01, respectively). Mortality rate was higher in men and anterior wall MI was found to be more prevalent than inferior wall MI (table 1).

Binary logistic regression analysis was used to determine the factors that affect mortality rate. It was shown that leukocytosis was an independent prognostic factor in patients with MI (P<0.001, exp (B)=23.03) (table 2).

Discussion

Ischemic heart disease and acute myocardial infarction (AMI) are among the most dramatic manifestations of cardiac diseases in the past few decades.[12]

Bae et al. concluded that combination of WBC, hemoglobin and platelet distribution width (PDW) are useful markers in early risk stratification in patients with AMI.[13]

A total of 404 patients who had undergone primary percutaneous coronary intervention (PPCI) showed that neutrophil/lymphocyte ratio was found to be associated independently with early infarct-related artery patency before PPCI in patients who have undergone PCI for ST-

Table 1: Demographical characteristics of the patients with MI

Characteristics	Total (n=106)	Deceased (n=15)	Survived (n=91)	P value
Age (year)	62.5±13.3	79.3±7.5	59.8±11.2	<0.001
Gender (male)	81 (76.4)	9 (60)	72 (79.1)	0.1
Gender (female)	25 (23.6)	6 (40)	19 (20.9)	
Anterior wall MI	64 (60.4)	8 (53.03)	56 (61.5)	0.5
Inferior wall MI	42 (39.6)	7 (46.7)	35 (38.5)	
WBC<10,000 /µl	78 (73.6)	2 (13.3)	76 (83.5)	<0.001
WBC>10,000 /µl	28 (24.4)	13 (86.7)	15 (16.5)	
Hb<13g/dl	41	10 (66.7)	31 (34.1)	0.01
Hb>13g/dl	65	5 (33.3)	60 (65.9)	

Table 2: Main determinants of short-term mortality in a multivariable binary logistic regression model

Characteristics	P value	Odds ratio	95% confidence Interval
Age [<60y vs ≥60y]	0.99	2.1	0.21-6.12
Gender [male vs female]	0.98	1.002	0.19-5.2
Leukocytosis [WBC<10000 vs WBC>10000]	<0.001	23.03	4.06-13.5
Anemia [Hb<13 vs Hb>13]	0.01	2.9	0.61-13.8
Location of MI [Anterior vs Inferior]	0.27	0.4	0.08-2.04

elevation myocardial infarction (STEMI). Therefore, these simple parameters can provide useful information on the related risk evaluation in these patients.[14]

Núñez et al. studied records of 1118 consecutive patients who were admitted with a diagnosis of AMI. WBC count was measured 24 hours following admission and All-cause mortality was recorded during a median follow-up period of 10+/-2 months. They concluded that WBC count on admission was an independent predictor of long-term mortality in AMI patients.[15]

Leukocytosis is reported to be associated with adverse hospital outcome in patients presenting with AMI. The association of this prognostic factor with hospital mortality and heart failure in patients with other acute coronary syndromes is unclear.[16] Furman et al. examined the association between admission leukocyte count and hospital mortality and heart failure in 8269 patients presenting with acute coronary syndrome and concluded initial leukocyte count is an independent predictor of hospital death and the development of heart failure.[16]

In another study on 585 patients with acute non-STEMI, blood leukocyte count was measured immediately after admission in the emergency department. Again leukocytosis on admission was an independent predictor of cardiovascular events in patients with acute non-STEMI.[17]

In a study investigating 152 patients suffering from ischemic heart disease (IHD) for up to 5-year follow-up, 1.8 times more mortality rate was observed in patients with leukocytosis (WBC>9000 /µl).[18] Another study also indicated a 10.4% higher mortality rate in patients with leukocytosis (WBC>15000/µl) within the first month of MI.[19]

To remove the effects of confounding factors such as sex and location of infarction, we did a logistic regression analysis. It was found that leukocytosis was an independent factor of mortality rate in the first 6 months following AMI. The death frequency was 20.4 times more common in patients with leukocytosis than deceased patients without leukocytosis. A previous study also demonstrated leukocytosis as an independent factor of mortality rate during a 10-year follow up investigation with a risk of 2.79, suggesting that a long term follow-up care of the patients with leukocytosis might reduce the mortality rate.[20] In current study, the association between mean WBC count and prognosis of the myocardial infarction was investigated during the first 48 hours and up to six months after onset of MI. The results showed that mortality rate was significantly associated with leukocytosis up to six-month follow-up period ($P<0.001$).

Conclusion

The findings of the current study showed that leukocytosis in patients with MI was significantly associated with higher rates of mortalities in short term follow up. Therefore, better care measurements and if necessary, performing invasive procedures, including PCI or coronary artery bypass grafting (CABG), can reduce mortality in these patients. Longer studies are required to provide precise information towards better understanding of the prognostic variables in patients with AMI.

Conflict of Interest: None declared.

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IJBC 2015; 7(5): 227-230

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

ORIGINAL ARTICLE

Evaluating Blood Requests and Transfusion Practice in Major Surgical Procedures

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ARTICLE INFO

Article History:
Received: 20.06.2015
Accepted: 10.09.2015

Keywords:
Blood transfusion
Red blood cell
Surgery
Cross match
Haemovigilance
Iran
Blood request and utilisation

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ABSTRACT

Background: Red blood cell and other blood products play a crucial role in management of different disease processes, but in spite of implementation of crucial steps to improve the process of request for blood and blood consumption, there is still not enough information available regarding the proper practice in many medical centres. We aimed to evaluate the status of blood product requests and transfusion practice in surgical patients in Al-Zahra and 17th Shahrivar hospitals, Rasht, Iran.

Materials: This retrospective, descriptive cross-sectional study was performed using medical records of patients undergoing major surgical procedures in Al-Zahra and 17th Shahrivar hospitals, Rasht, Iran, from April to December 2013. The cross-match to transfusion ratio (C/T ratio), transfusion probability (T%) and transfusion index (TI) were analyzed. We used SPSS analytical software to analyze the data.

Results: Transfusion index was 0.27, transfusion probability 12.8% and C/T ratio was 7.38 which were higher than standards, indicating that only 54 units out of 399 red blood cells units requested were used. Laparoscopic surgery had the worst indicator in terms of wastage of packed cell products. These findings in 17th Shahrivar hospital were 0.09, 8.82% and 12.5, respectively. In this hospital only 10 units out of 125 requested units were used. Appendectomy showed the worst indicators.

Conclusion: Blood transfusion indexes particularly for laparoscopic and appendectomy procedures were high in two hospitals studied. In order to overcome this problems, providing teaching courses for proper transfusion practice in surgery departments to improve their knowledge of haemovigilance and preparation of defined guidelines for red blood cells' cross matching and transfusion is recommended.

Please cite this article as: Sheykhansari Charvadeh S, Darbandi B, Zahiri Sorouri Z, Baghersalimi A. Evaluating Blood Requests and Transfusion Practice in Major Surgical Procedures. IJBC 2015; 7(5): 227-230.

Introduction

In the first half of the twentieth century and with discovery of blood groups (ABO and Rh) and also with improvement of conservative materials for preservation of blood products, the ability to use human blood as a vital component in medicine for treatment of patients with severe disease was achieved. Blood transfusion is an important life-saving component in daily clinical practice, specifically in major surgeries. However, due to resource constraints, it is not always possible for the blood product to reach the patient at the right time,[1] as a result the proper use of these vital products is one of the main challenges in blood transfusion practice. In our country, despite having the volunteer-based nature of

blood donation, preparation of blood and its byproducts needs a high amount of financial resources including the cost of instruments, well organized laboratories and specialized personnel in this process. Because of this high cost process the appropriate use of blood products holds an important and undeniable priority.[1]

Increased blood request by physicians and inappropriate use of blood products cause a serious loss of financial and human resources, which indicates the need for performing research on means to improve the ordering and consumption of blood products.[2]

The high rate of ordering of blood might be related to the fear of health care personnel about the consequences of unavailability of blood products during the surgery, lack of knowledge regarding the amount of accessible blood in blood banks, or absence of appropriate guidelines for blood transfusion.[3-5] The goal of the present study was to evaluate the process of packed red cell ordering and its consumption based on the kind of surgery performed in two medical surgery centres in order to reach a consensus recommendation.

Patients and Methods

This retrospective study was performed in Al-Zahra and 17th Shahrivar hospitals in Rasht, North of Iran. Medical records of 377 patients hospitalized from April to December 2013 were evaluated. Hospitals' blood bank records, cross-match requests for hospitalized patients in obstetrics and gynecology departments of Al-Zahra Hospital and pediatric surgery department of 17th Shahrivar Hospital were studied and the results were filled in a predesigned questionnaire. Then blood units which were actually consumed based upon the respective department and kind of surgery wrere extracted. Three main indicators of blood transfusion consumption were calculated as follows:[6]

1-Cross match to transfusion ratio (C/T ratio): This index is the most important parameter for estimating the need for blood during surgery. A C/T ratio lower than 2.5 is a significant indicator of the need for blood transfusion during surgery.

2-Transfusion probability (T%): It indicates the probability of requirement of the blood during a surgery and a value greater than 30% indicates considerable requirement of blood.

3-Transfusion index (TI): It shows the average number of units used per patient and for operations that usually require less than 0.5 units of blood, blood compatibility testing before surgery is not necessary.

SPSS analytical software version 16 (Chicago, Illinois, USA) was used to perform the statistical analysis. P values less than 0.05 were considered statistically significant.

Results

Medical records of 377 patients (269 and 108 patients from Alzahra and 17th Shahrivar Hospital, respectively) were analyzed. Mean age of adult patients was 31.83±9.7 years and mean age of pediatric patients was 4.48±4.23 years (table 1).

The main surgical procedure performed in Al-Zahra Hospital was caesarean section followed by laparotomy, laparoscopy, hysterectomy and myomectomy. Surgical procedures in 17th Shahrivar hospital were appendectomy, herniorrhaphy, laparotomy, orchiopexy, and intussusception.

Table 1: The age distribution of patients in two medical centers

17th Shahrivar Hospital	Al-Zahra Hospital
Age category number (%)	**Age category number (%)**
Less than 1: 40 (37)	Less than 20: 19 (7.1)
1-3: 15 (13.9)	21-30:117 (43.5)
3-7: 23 (21.3)	31-40: 98(36.4)
More than 7: 30 (27.8)	41-50: 23 (8.6)
	More than 50: 12(4.5)
Total: 108	Total: 269

In Al-Zahra Hospital, 399 packed cell units were cross-matched with only 54 units being transfused. The C/T ratio in this centre was 7.38. The highest and lowest C/T ratios were 41 and 2.46 which belonged to laparoscopy and hysterectomy, respectively. For 108 pediatric patients from 17th Shahrivar Hospital, 125 packed cell units were cross-matched with only 10 units being transfused. The total C/T ratio in this population was 12.5 with the highest C/T ratio of 56 for the appendectomy and lowest ratio of 3 for the laparotomy. The total transfusion probability (T%) for Al-Zahra Hospital was 12.8 % with the lowest T% of 3.1% for laparoscopy and the highest T% of 33.3% for hysterectomy. The total T% for the 17th Shahrivar Hospital was 8.82% with the lowest T% of 2.6% for appendectomy and highest T% of 33.3% for intussusception. The transfusion index (TI) for 269 patients in Al-Zahra hospital was 0.27 with the lowest TI of 0.03 for laparoscopy and highest of 1.08 for hysterectomy. TI index for patients in 17th Shahrivar Hospital was 0.09 with the highest TI of 0.44 for intussusception and the lowest TI of 0.026 for appendectomy. The details are presented in table 2.

Discussion

Most previous studies showed higher need for cross-match versus world standards.[5-7] Also, similar Iranian studies showed a much higher rate of cross-matching compared to world standards. It seems that this may be the result of unfamiliarity of health care professionals with the standards of transfusion practice and blood ordering or lack of a proper national guideline or the concern of medical staff regarding access to blood products in emergency conditions.[1,3,4,8]

Three main indices used in ordering blood products including C/T ratio, transfusion probability and TI were evaluated in the present study. We found that the C/T ratio for the Al-Zahra hospital was 7.38 indicating that only 12.8 percent of cross-matched packed cell units were actually transfused. Also among different surgical procedures performed in this centre, the C/T ratio for hysterectomy and laparotomy were near standards but it was much higher than standards for other procedures.

In 17th Shahrivar Hospital, the total C/T ratio was 12.5 and TI was 0.09 indicating that only 8.82 percent of cross-matched packed cell units were actually

Table 2: Transfusion indexes based on related surgeries and the medical center.

	TI	T%	C/T ratio	Number
Al-Zahra Hospital				
Cesarean section	0.11	6.6	18.9	179
Laparotomy	0.67	30	3.22	41
Laparoscopy	0.03	3.1	41	32
Hysterectomy	1.08	33.3	2.46	12
Myomectomy	0.2	20	12	5
Total	0.27	12.8	7.38	269
17thSharivarHospital				
Appendectomy	0.026	2.6	56	41
Herniorrhaphy	0.027	2.7	38	38
Laparotomy	0.4	30	3	11
Orchiopexy	0.12	12.5	9	9
intussusception	0.44	33.3	2.5	9
Total	0.09	8.82	12.5	108

transfused. In this center, except for intussusception and laparotomy which showed C/T ratios near the standard, the C/T ratio for other procedures was much higher than world standards.

In a study by Abbasyvash et al.,[1] the C/T ratio was reported to be 7.8 and the total blood transfusion index was reported to be 0.25 which was much higher than standard blood ordering except for few surgical procedures similar to our findings.

In a study by Khoshrang et al. C/T ratio, T% index and TI index were reported to be 14.18, 8.85% and 0.11, respectively.[6] Our figures were closer to standards in comparison to their study which could be attributed to higher risk surgical procedures in their study or more logical practice of our practitioners.

The problem of higher than standard cross-match ordering has also been reported in other countries. In a study by Benset et al.,[5] a C/T ratio of 7.3 has been reported which is very near to the ratio that we found in Al-Zahra hospital. In studies done by Iwasaki et al.[9] and Mahar et al.,[10] the results were closer to standards rather our findings. The C/T ratio in these two studies were 1.71 and 1, respectively which could be attributed to their proper use of MSBOS guidelines.

Sajwani et al. have reported a C/T ratio of 1.6 in their study which is again much better than our finding and can be credited to the educational classes for improving the knowledge of their practitioners regarding appropriate blood ordering practices.[11]

All these findings showed that considering the standard cross-match indexes of C/T ratio of 2.5, TI>0.5 and T%>30% in blood transfusion practice, cross match ordering has been much higher than standards in many centres.

We also found that our cross-match orderings in Al-Zahra and 17th Shahrivar Hospitals were much higher than world standards indicating that doing cross-match in many surgical procedures were performed unnecessarily in our centres.

Conclusion

Blood transfusion indexes particularly for laparoscopy and appendectomy were high in our studies. In order to resolve this problem, providing teaching courses for medical surgical staff to improve their knowledge of hemovigilance procedures and preparation of consent guidelines for red blood cell cross-matching is recommended.

Conflict of Interest: None declared.

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IJBC 2015; 7(5): 231-234

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

CASE REPORT

Metronomic Maintenance Therapy in Refractory Acute Myeloblastic Leukemia with Monosomy 7

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ARTICLE INFO

Article History:
Received: 25.05.2015
Accepted: 30.08.2015

Keywords:
Metronomic Chemotherapy
Histone deacetylase Inhibitor
Acute Myeloblastic Leukemia
Valporic acid
All-trans retinoic acid

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ABSTRACT

Patients with acute myeloblastic leukemia (AML) with monosomy 7 are a group of patients with refractory AML who have a very poor prognosis. Therefore, rationally designed new therapies, including metronomic chemotherapy regimen with histidine deacetylase inhibitors (Valporic acid, ATRA) are being investigated as potential treatments for the population of refractory cases of AML. Herein, we report a patient with primary refractory AML who was treated with oral low-dose chemotherapy after standard systemic chemotherapy.

Please cite this article as: Shahriari M, Abdolkarimi B, Saleh F, Salajeghe P. Metronomic Maintenance Therapy in Refractory Acute Myeloblastic Leukemia with Monosomy 7. IJBC 2015; 7(5): 231-234.

Introduction

Hematological malignancies are usually primarily treated by systemic chemotherapy. Induction failure is still a dismal event in acute leukemia especially in those with chromosomal abnormalities such as monosomy 5 and 7. These patients often have low disease free survival rate after stem cell transplantation. Multidrug resistance is a major cause of treatment failure and death in them.[1]

Despite receiving combination chemotherapy, treatment failure and relapse occurrs in more than half of the cases with acute myeloid leukaemia (AML).[1] A few therapeutic strategies are recognized for treatment of AML to improve survival in patients with recurrent chromosomal abnormalities.

Inhibition of histone deacetylases (HDACs) with continuous low-dose all-trans retinoic acid (ATRA) and Valporic acid as differentiating agents has been proposed as alternative treatments in AML during the last decade. It is being used as metronomic chemotherapy which is intended to prevent tumor angiogenesis and induce apoptosis in myeloid blasts.[2] Herein, we report a patient with primary refractory AML who was treated with oral low-dose metronomic therapy after standard systemic chemotherapy.

Case Presentation

A 13-year-old Iranian boy was admitted to our hospital with pallor, fever and lethargy since 1 week previous to admission. Physical examination showed low grade fever, pallor without any neurologic sign, hepatosplenomegaly

or lymphadenopathy. CBC showed hemoglobin 3.5g/dL, platelets 64×10^9/µL, and leukocytes 55×10^9/µL with 4% blasts, 8% neutrophils, 80% lymphocytes, 6% monocytes and 2% eosinophil. Bone marrow aspiration showed 75% of total nucleated cells had myeloblastic phenotype (figure 1). Immunophenotyping analysis by flow cytometry was performed by a panel of antibodies. The blast cells were positive for CD45, CD13, CD117, CD34, CD19, HLA-DR and negative for other lymphoid markers including CD5, CD10 and also CD14. Negative controls were assessed by IgG1FITC/IgG1PE. Therefore, the patient was diagnosed as AML FAB-M_1 with aberrant expression of CD19 (figure 1). Karyotype study showed 45, XY,-7 compatible with monosomy 7 (figure 2).

The patient was considered a candidate for allogenic bone marrow transplantation after achieving first induction remission. He was initially treated by a course of MRC-12 protocol: Adriamycin (33.5 mg /m^2 , days 1,3,5), Cytarabine Arabinoside (100 mg /m^2 , days 1 to 10) and Etoposide (100 mg /m^2 , days 1 to 5) was the first course of induction phase which was not successful (induction failure) and then he was scheduled for 1st and 2nd course of FLAI protocol (Fludarabine (25 mg/m^2, days 1–4), Cytarabine Arabinoside (1000 mg/m^2, days 1–4) and Idarubicin (5 mg/m^2, days 1–3). However, he had no response to either 2 courses of FLAI protocol and repeated bone marrow aspiration and biopsy showed 60% myeloblast in bone marrow specimen. Then the patient was treated by alternative chemotherapy protocol including 5-day course of Cladribine (9 mg/m^2/dose) and Cytarabine Arabinoside as daily 2-hour infusions (500 mg/m^2/dose). But again he had no response to 1st course of the alternative protocol. Finally after explaining the situation for the patient and his parents, he was scheduled for our target regimen as oral metronomic chemotherapy in which Histone Deacetylase Inhibitors

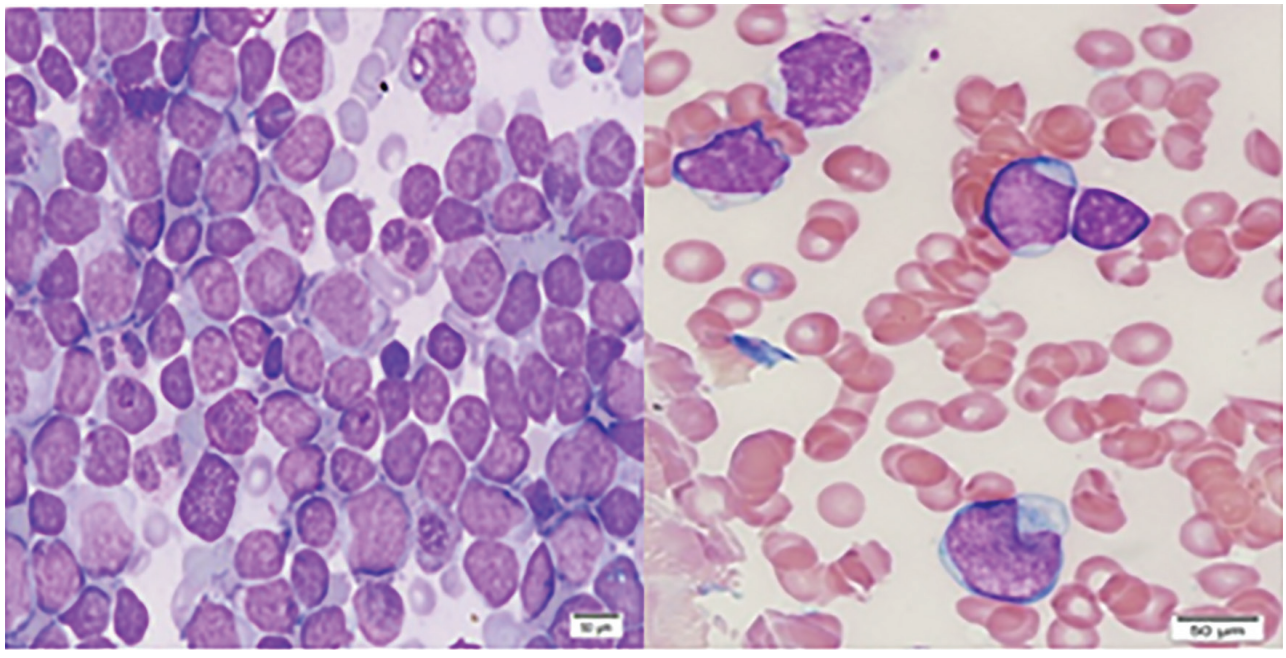

Figure 1: A) Bone marrow aspiration (left) showed blasts positive for myeloperoxidase, Sudan black, and non-specific esterase and were sensitive to fluoride inhibition. B) Peripheral blood showed myeloblasts.

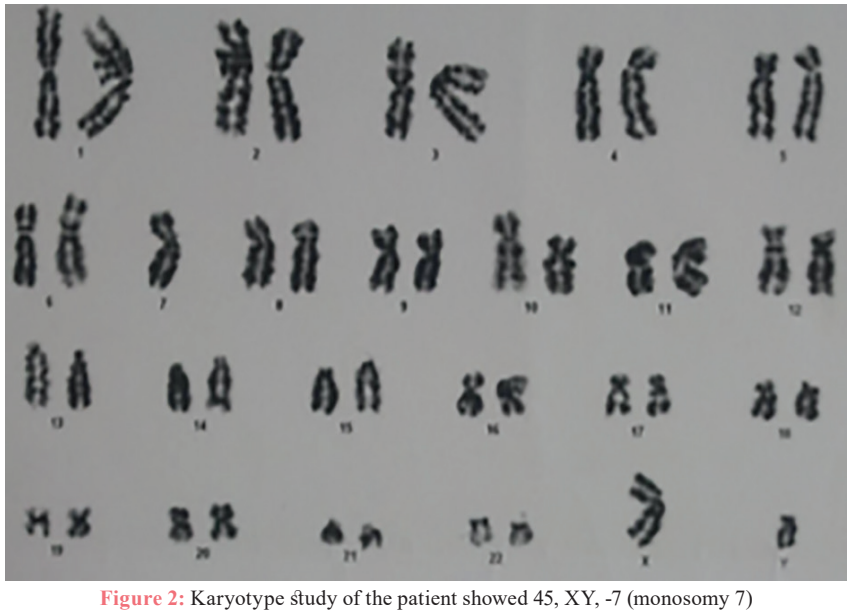

Figure 2: Karyotype study of the patient showed 45, XY, -7 (monosomy 7)

(HDAI) were included: 6-Thioguanin 40 mg/m^2 for 21 days, Prednisolone 40 mg/m^2 for 5 days, oral Etoposide 50 mg/m^2/day for 21 days plus ATRA 45mg/m^2/day and Valproic acid 2.5-5 mg/kg/day for the first 14 days of month followed by 7 days of rest.

Now after 1 year since beginning of metronomic chemotherapy, he occasionally receives only supportive care such as antibiotics and transfusions of blood products; however, there is no evidence of remission in bone marrow and peripheral blood smear. The patient did not experience any adverse event related to treatment with ATRA (dryness of mucosa, headache and increased transaminases or triglycerides).

Metronomic chemotherapy as maintenance was continued for the patient due to lack of response to standard systemic chemotherapy in order to to prolong survival and improve patient's quality of life.

Discussion

Metronomic chemotherapy is continuous systemic administration of non-toxic doses of drugs that attack proliferating endothelial cells as targets during tumor angiogenesis. This strategy was innovated 40 years ago in adult oncology, but experiences in pediatric oncology are scanty especially in hematological cancers. This strategy often is used in management of solid tumors by co-administration of anti-angiogenic drugs plus low-dose continous chemotherapy instead of high dose intermittent chemotherapy.[3]

Currently, PrET protocol (Prednisolone, Etoposide and Thioguanine) is a well-known metronomic regimen for refractory AML patients. The anti-angiogenic ability of 6TG, together with its antimetabolite activity toward tumor cells has a major role supporting efficacy of this method.[4]

In one study, a 68-year-old man was reported who had AML with high-risk cytogenetic features such as our patient who achieved complete remission during induction phase by oral metronomic chemotherapy by similar regimen in an outpatient settings. He was then treated with high-dose cytarabine Arabinoside (HDAC) as consolidation followed by maintenance therapy with PrET protocol. He was survived 35 months since diagnosis and 21 months off treatment.[5]

Our patient did not achieve remission despite different kinds of salvage regimens he received (FLAI, Cladribine and HDAC). As a result, he was determined to be treated by PrET protocol as palliative treatment. Two components of our treatment were administration of Histone deacetylase inhibitors (HDACI) including valporic acid (VPA) and All-trans retinoic acid (ATRA) that were co-administered as metronomic chemotherapy.

Valporic acid (VPA) has antileukemic effects in AML used in combination with other antileukemic agents.[4] This treatment can induce a clinically relevant improvement in peripheral blood cell counts and stabilization of the clinical status for a subset of AML patients, as well as reducing the risk of clinically relevant toxicity. Although our patient had a stable clinical status, the most cell population in peripheral blood and bone marrow was myeloblasts.

It seems that VPA could induce differentiation and has anti-proliferative and pro-apoptotic effects in AML cell lines. However, patients are most likely heterogeneous in terms of susceptibility to VPA and molecular mechanisms mediating its antileukemic effects. Direct effect of the drug on leukemic cells seems to be the most important, but there may be indirect effects mediated through increased antileukemic immune reactivity.[6,7]

ATRA is also a HDAC inhibitor which its differentiating effect on human acute promyelocytic leukemia (APL) cells in vitro has been well established.[7] In APL, absence of ATRA leads to HDAC activities, inducing chromatin condensation and transcriptional repression.[4] ATRA induces a conformational change in the promyelocytic leukemia (PML)/retinoic acid receptor α (RARα) fusion oncoprotein, thereby allowing the release of HDAC complexes and recruitment of transcription. Treatment with ATRA has dramatically improved prognosis of APL and has also been used in the treatment of non-APL AML.[2]

We used combination of PrET metronomic chemotherapy along with ATRA and VPA after failure of various intensive salvage protocols for this patient, since he had no chance for continuation of treatment and prolonged survival; however, this method of led to improved survival and increased quality of life.

Synchronous prescription of ATRA and VPA can be combined with low-dose cytotoxic drugs such as Cytarabine Arabinoside.[8] Hydroxyurea and 6-thioguanine,[6] can also induce remission according to the Myelodysplastic Syndrome (MDS) response criteria and complete hematological remission. Our patient showed evidence of clinical stability with metronomic strategy despite lack of signs of remission induction on bone marrow. Satisfactory results with other combinations of oral metronomic therapies such as melphalan and lenalidomide but without HDAC inhibitiors has been reported.[9-12] It seems this method could induce a lifesaving dormancy condition in the patient preventing from flare up of the primary disease.

Conclusion

Metronomic chemotherapy with HDAC inhibitors can be employed as a therapeutic strategy particularly in refractory AML cases not responsive to other treatments. This case report suggests the probable efficacy of combination of oral low-intensity metronomic chemotherapy by HDAC inhibitors in AML patients with induction failure.

Conflict of Interest: None declared.

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IJBC 2015; 7(5): 235-236

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

Letter to Editor

Disseminated Intravascular Coagulation in a Case of Brucellosis Misdiagnosed as Thrombotic Thrombocytopenia Purpura

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ARTICLE INFO

Article History:
Received: 25.05.2015
Accepted: 30.08.2015

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Please cite this article as: Valizadeh N. Disseminated Intravascular Coagulation in a Case of Brucellosis Misdiagnosed as Thrombotic Thrombocytopenia Purpura. IJBC 2015; 7(5): 235-236.

Dear Editor

Brucellosis is a multisystem disease with various clinical presentations. It is a worldwide, zoonotic disease which has still remained as an uncontrolled health problem in many underdeveloped countries.[1] Hematological manifestations of brucellosis are so variable and include anemia, leukopenia, immune thrombocytopenia, thrombotic thrombocytopenia purpura (TTP) and hemophagocytic syndrome.[2-7] Disseminated intravascular coagulation (DIC) has been reported in a few cases of brucellosis.[8-10] Herein, we present a case of brucellosis who was presented with clinical picture of DIC misdiagnosed as TTP.

A 24-year-old woman was admitted with fever, abdominal pain and headache for the last three months. She was from a rural area in east Azarbaijan of Iran and had a history of fresh dairy consumption. On physical examination she had pallor, fever, and jaundice. Laboratory tests showed anemia (Hb: 7.6g/dl), thrombocytopenia (Platelet count 30×10^9/µl), indirect hyperbilirubinema, and elevated lactate dehydrogenase (LDH). Peripheral blood smear revealed many fragmented RBCs. A diagnosis of TTP was made and urgent plasmapheresis was initiated for the patient, but the results of further tests in the next few days showed prolonged prothrombin time (PT), increased fibrin degradation products (FDP) and D-dimer along with markedly elevated anti-brucella antibodies with Wright's *and Coombs wright's* tests, thus the diagnosis of DIC secondary to brucellosis was affirmed. Treatment with doxycycline and rifampin was initiated for the patient. Three days following appropriate antimicrobial therapy thrombocytopenia and coagulopathy were resolved and she was discharged with anti-brucellosis medication.

Although TTP is characteristically defined by a pentad of thrombocytopenia, microangiopathic hemolytic anemia, fluctuating neurological signs, renal impairment and fever, it can be considered without fulfilling the whole pentad. In another word positivity for only the first 3 above mentioned items could be present in approximately 75% of the cases.[5] TTP is a thrombotic microangiopathy similar to DIC; however, in contrast to DIC, the mechanism of thrombosis in TTP is not via the coagulation pathway activation and consumptive coagulopathy. The results of the blood coagulation assays (PT, PTT, D-Dimer, FDP and fibrinogen levels) in TTP were normal in our patient.

According to the presence of fever, headache,

microangiopathic hemolytic anemia and thrombocytopenia, diagnosis of TTP was made for our patient, but further laboratory studies indicating prolonged PT, increased FDP and D-dimer was compatible with the diagnosis of DIC secondary to brucellosis.

There are various hematological manifestations in brucellosis which include anemia, leucopoenia, thrombocytopenia, TTP, hemophagocytic syndrome and rarely DIC.[2-9] As a result, in patients with brucellosis presenting with abnormal hematological features such as DIC, diagnosis of the disease may be problematic and delayed, accordingly we should consider brucellosis in any patient with unexplained DIC in endemic areas for brucellosis.

Conflict of Interest: None declared.

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