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Open Access Editorial

Scientific Conferences: Should we go all virtual?

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COVID -19 pandemic led to a rapid transition in human adaptation. Teaching and learning have transformed from a physical mode to a virtual mode. In this situation, the Pakistan Society of Haematology (PSH) decided to hold its Haemcon in virtual mode. President PSH and Chairperson of the Scientific Committee with the cooperation of their team and haematologists put-in all the efforts to accomplish this enormous task in a perfect manner, since it was the first ever experience of holding international virtual conference by any professional society in Pakistan. Their persistent efforts had a fruition by holding multiple sessions involving haematologists across the country. International speakers also contributed, by sharing new updates and by presenting research papers. The whole content is now available online. After taking the stock of it, it can easily be claimed that this alignment by PSH from physical to virtual platform is well thought and was executed seamlessly. Hopefully it will go a long way, in ensuring continuous professional development and education for all in the wake of continuous covid 19 pandemic.

For virtual sessions it hold true that they can be for anybody, anywhere in the world, as long as they have an internet connection. One can listen to these live, but can also be listened later on, as content is uploaded on YouTube. This more visibility is leading to availability of quality content, as one cannot afford the risk of having lesser viewers and more dislikes. Virtual mode allows people with conflicting schedules, living in different time zones or non-availability due to personal commitments, to benefit equally. Different features of virtual activities make these more egalitarian, inclusive and environmentally friendly (less carbon foot prints), than in-person seminars or conferences. Audience can be very large, which makes virtual events more inclusive. By removing barriers, virtual conferences make it easy to, to access knowledge regardless of financial status, family obligations, sexual inequalities or geographical locations. Going virtual have removed the obstacles of diplomatic and travelling restrictions, finances, being absent from the work place and many more. Surveys revealed that majority of the professionals think that scientific meetings should continue to be virtual or have a significant virtual component, even after the pandemic.

The disadvantages of physical sessions are the high cost, enormous cumulative time invested by the delegates, a large carbon foot print and risk of disease transmission in the wake of ongoing COVID-19 pandemic. With virtual activities, carbon footprints originating from travel witnessed a substantial decline. In USA it is calculated that even if 50% of weekly seminars went virtual, that would save around 30 tons of emission. Organizers of two fully virtual conferences in USA estimated that their total carbon emissions were less than one percent of a traditional physical or fly-in event. Over the time fun, flair and food is on the higher side in the conferences. In virtual happenings all this is curtailed to a larger extent. Physical events are much more resource intensive. They tend to include enormous showbiz style social events.

Virtual conferences can be made more productive by having shorter symposiums sessions over the span of extended days, rather than packed into a small time span. Virtual conferences witness more democratic question and answer sessions, as contrast to in-person events where few people dominate. The wider discussion can be generated by creating #hashtags on twitter with specific themes or topics. The Twitter discussions allow asynchronous questions and answers, which benefit attendees from different time zones, and provide a permanent record.

During the past two decades Pakistan has seen around 70% female representation among health professionals. This has made physical attendance and travel a bit challenging for some professional and the virtual CME activities are well poised to cater for this tier.

Being virtual is very much in fashion, but it is required to address the issues of accessibility, zone-time differences, technological training and glitches, internet bandwidth issues, regulations and standard operative procedures still not time tested, digital burn out and many others. The initial enthusiasm may lead to "zoom-exhaustion", which is already being reported. Less research papers submission has been documented for virtual conferences. How to keep people involved and attentive in virtual milieu is required to be catered for. Virtual conferences don't allow researchers to retreat from their everyday routines and fully immerse themselves in knowledge sharing. Face-to-face interactions and developing acquaintances in the corridors and during leisure time during sessions will be missed. Sponsors are likely to lose interest. Trouble shootings are not so infrequent and there is requirement to make sure throughout the activity that the remote attendees are on line.

A plausible solution lies in being able to organize purposeful, carefully planned, environmentally sustainable and financially conservative hybrid event, having physical and virtual components. To be stand still is against human and intellectual advancement. Knowledge and practice always need to be underpinned by the technology of the day. So to serve and survive, we are required to keep pruning, expanding and refining the skills.

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Open Access Review Article

The Utilization of Mesenchymal Stem Cell Therapy in the Treatment of Human Diseases

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MSC is the most important type of adult stem cells. These are multipotent and can be obtained from various sources, including adipose tissue, bone marrow, liver, and cord blood. MSCs are capable of self-renewal and could differentiate into various cell types. MSCs can be grown in-vitro, and these are known to execute anti-inflammatory, anti-fibrotic, anti-tumor, anti-apoptotic, and immunomodulatory effects. Many studies have reported that autologous or allogenic administration of MSCs to treat various diseases is relatively safe. Following transfusion, MSCs show low immunogenicity. MSCs could travel to inflammatory or damaged tissue sites. These could also reach the tumor site and home there and contribute to suppression of tumor growth. In this review, we have summarized the important properties of MSCs, their role in the treatment of cancer, immune diseases, and regeneration of damaged tissues. The information about the application of MSCs in different therapeutic strategies may help better understand the pros and cons of MSCs therapy in human diseases.

Key words: Cancer, Drug delivery, Immune system, MSC, Regenerative medicine.

Introduction

Embryonic stem cells and adult stem cells are the main types of stem cells. The pluripotent nature of embryonic stem cells enables these cells to form embryonic structures while adult stem cells could undergo self-renewal and transform into various cell types. Mesenchymal stem cell (MSC) is one of the main types of adult stem cells. [1, 2] These were first discovered in 1970 by Friedenstein. MSCs can be obtained from multiple sources, including bone marrow, fat, umbilical cord tissue and blood, muscle tissue, amniotic fluid, etc. Figure 1. [3, 4]

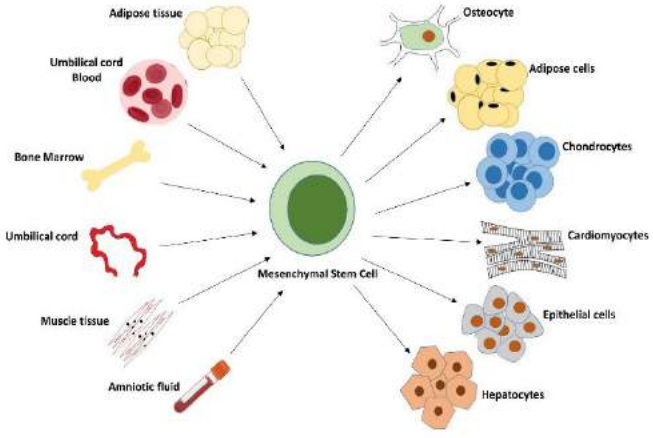

Figure 1. Different sources of MSCs and common MSCs differentiated cell types.

The biological properties of MSCs could be different as per their tissue source, but there are some important characteristics that MSCs, from any source must exhibit. The International Society for Cellular Therapy (ISCT) described these characteristics as follows: When MSCs are grown in-vitro, these must adhere to plastic surfaces. There must be the expression of CD73, CD90, CD105 surface antigens and absence of expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, HLA-DR surface antigens on the surfaces of MSCs.[5] Under directed cell culture conditions, these must show differentiation into mesodermal cells like adipocytes, chondrocytes, osteoblasts, and ectodermal or endodermal cells like neuronal cells, cardiomyocytes, hepatocytes, or epithelial cells. [6, 7]

Currently, MSCs are the most common cells being used in cell-based therapeutic approaches. Important advantages like MSCs can be acquired easily from multiple sources; these can be grown in-vitro at large scales, these exhibit low immunogenicity, and excellent differentiation capability make them favorite candidates to be used in cell-based treatment approaches.[8,9] Allogenic and autologous MSCs transfusions have been successfully administered in many studies. MSCs have been utilized in several clinical studies to treat various human pathological conditions like renal, cardiovascular, neurological, hepatic, and lung disorders.[10-13] These have also been employed in regenerative medicine and oncology to repair damaged tissues and treat cancer.

MSCs have also shown potent immunomodulatory effects. These could secrete a variety of immune mediators, which could interfere with cellular signaling mechanisms and contribute to achieving therapeutic effects in immune diseases.[14, 15] Given the abilities mentioned above, it can be summarized that MSCs are strong tools for cell-based therapies to treat various human diseases.

Salient biological features of MSCs

Besides owning auto-renewal and multidirectional differentiation potential as other stem cells, MSCs are associated with other unique properties. MSCs could interact with immune cells to modulate immune responses; these could also participate in paracrine signaling. Additionally, MSCs possess the characteristic of poor immunogenicity. Therefore, immunological rejection is less likely to occur during cellular therapy with MSCs.

Moreover, MSCs can move towards locations of inflammation and tumors in the body and could settle there. Therefore, based on the characteristics mentioned above, MSCs have significant potential in treating age-related or pathological damage to tissues. These could act as an excellent means of replacement cells for repairing age or disease-related damage to tissues and organs. MSCs could offer broad-spectrum therapeutic benefits in the treatment of Inflammatory, autoimmune disorders, and cancer.

The ability of MSCs to regulate the immune system

MSCs could suppress the growth of natural killer cells and attenuate their cytotoxicity by releasing the prostaglandin E2 (PGE2), soluble human leukocyte antigen G5 (sHLA-G5), and indoleamine 2,3-dioxygenase (IDO).[16] MSCs could also interfere with the differentiation of monocytes into dendritic cells and suppress the growth of dendritic cells.[17] MSCs, through the release of PGE2, could attenuate TNF gene expression and enhance IL-10 expression by dendritic cells.[18, 19] MSCs-mediated modulatory effects not only affect the differentiation and maturation of DCs. MSCs also interfere with their antigen-presenting ability to T lymphocytes, resulting in immunosuppression.[20]

Additionally, MSCs secrete significant quantities of interleukin 6 (IL-6), which helps minimize respiratory outbursts and provide protection to neutrophils. [16]

MSCs also play their modulatory role in the acquired immune system. MSCs secrete high quantities of PGE2, IDO, TGF-b1, HGF, iNOS, and HO-1, which interfere with the proliferation and maturation of CD4 T lymphocytes. Consequently, the maturation of B lymphocytes and the generation of antibodies are affected. [21, 22]

MSCs could induce immune tolerance. HLA-G5, secreted by MSCs, suppresses the cytotoxic activity of CD8 T lymphocytes and enhances the propagation and maturation of regulatory T cells. Which then act to maintain homeostasis and immune tolerance.[23, 24] Additionally, several research investigations have shown that during the state of inflammation, MSCs can transform the pro-inflammatory activity of macrophages to anti-inflammatory activity. MSCs could drive such transition by secreting TNF-a-stimulating gene 6 (TSG-6), PGE2, and IDO. These immune mediators act on macrophages to alter their immune-regulatory role from immune activators to immune-suppressors Figure 2A .[25]

MSCs induce minimum immune responses

Literature review indicates that exogenous MSC transfusion suppressed the activation of lymphocytes and prolonged the skin transplantation survival time.[26] Another study reported that allogeneic, semi-compatible, and mismatched bone marrow-derived MSC transplants may successfully treat GVHD, suggesting that stringent compatibility was unnecessary for treating GVHD. These findings indicate that low Immunogenicity of MSCs is important for the success of allogeneic MSC transplantation in preclinical and clinical usage.[27, 28]

Studies have demonstrated that MSCs constitutively express the MHC class I (MHC I). [29] The expression of MHC 1 is important because it shields MSCs from the harmful actions of NK cells.[30] MSCs also express HLA-G, which is an MHC-like protein. HLA-G proved to lower the chances of NK-mediated rejection following allogeneic MSC transplantation. [31, 32]

Furthermore, activated or resting MSCs do not promote the propagation of peripheral blood mononuclear cells (PBMCs) in the resting state and do not trigger PBMC propagation, which is responsible for inducing an inflammatory response. This finding further suggests that activated or resting MSCs exert minimum immunogenicity. [33]

Immune mediators secreted by MSCs like IL-10, TGF-b1, and PGE2 could effectively suppress the propagation of PBMCs and cytotoxic activities of T cells.

The abovementioned findings indicate that allogeneic MSC transplantation can be carried out with no or minimum host immunosuppression Figure 2B.[34, 35]

The migratory and homing abilities of MSCs

MSCs can move towards the site of lesions in various health conditions like tissue damage, inflammation, and tumors. The migration and settling down of MSCs at the lesion site are called homing.[36] Secretion of various cell adhesion molecules by MSCs and expression of chemokine receptors at their surfaces enable MSCs to carry out homing.[37] Like leukocytes, MSCs follow activation, adhesion, and migration steps to complete homing.[38] Inflammation or tissue damage causes the release of specific cytokines, which activate the vascular cell adhesion molecule-1 (VCAM-1) and α4β1 integrin (VLA-4) on the surfaces of endothelial cells and MSCs, respectively.[38, 39]

Besides cytokines, inflammatory and damaged tissues sites also trigger the secretion of growth factors like Hepatocyte growth factor (HGF), stromal cell-derived factor-1 (SDF-1), macrophage inflammatory protein-1 (MIP-1), and hyaluronic acid (HA). These growth factors bind to related receptors on MSCs and facilitate the MSCs-endothelial cells adhesion.[40, 41] Further, the secretion of matrix metalloproteinase 2 (MMP-2) and membrane type-1-MMP (MT-1-MMP) by MSCs cause degradation of extracellular material and let MSCs cross basement membrane and reach the injured site.[42]

MSCs could adhere to vascular endothelium through active capture or passive capture process.

Through the active capture method, the movement of MSCs towards vascular endothelial occurs through capillaries. In this method, the movement of MSCs is smooth and is in the direction of blood flow.[43] Once MSCs reach the vascular endothelium close to damaged sites, these are activated and migrate through the vascular endothelium to the inflammatory or damaged site Figure 2C. [44, 45]

While in the passive capture method, the movement of MSCs is through is not smooth. Due to their large size, their movement is hindered in capillaries. As a result, these are squeezed by the capillary wall and passively lodged inside endothelial cells. This course of action might affect the direction of blood flow and further contribute to capillaries' blockage.[46] Furthermore, in the passive capture process, MSCs could end up at a damaged site, or their route could also deviate to normal organs like lungs.[33, 35] This fact urges the importance of the careful selection of cell concentration and intravenous transfusion method during cell therapy with MSCs.

The use of MSCs in the treatment of human diseases

The possession of multipotent differentiation capacity by MSCs makes them a suitable candidate for their use in cell-based therapies. Further, the immunomodulatory

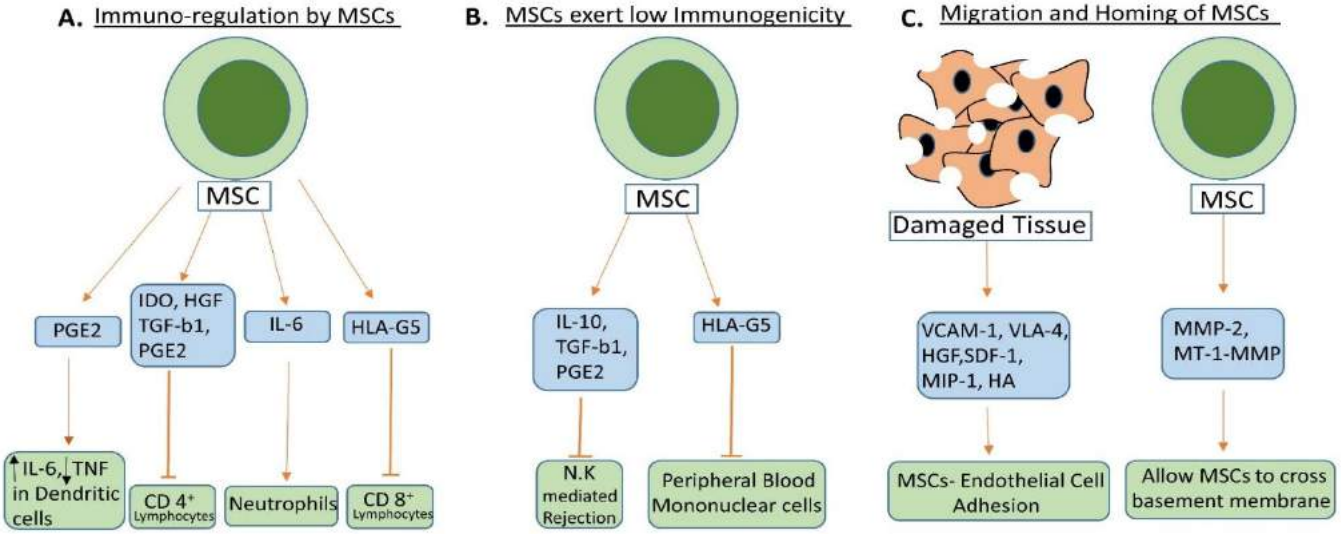

Figure 2. Important Biological functions of MSCs

effects, low immunogenic effects, and the ability to home at damaged tissue site support their utilization in multiple therapeutic strategies. [34]

The therapeutic effects of MSCs in regenerative medicine

One of the essential components of Regenerative medicine is stem cell therapy. In regenerative medicine, the therapeutic effects of stem cells are achieved by delivering stem cells to the damaged tissue site. Following transfer to the damaged site, the differentiation, regenerative abilities of stem cells, and the activation of paracrine signaling by stem cells contribute to healing tissue damage and restoring altered tissue functions. [47, 48]

MSCs therapy is considered a practical approach to treat disease in regenerative medicine. MSCs can be isolated from multiple sources; these are well capable of proliferation and differentiation. Most importantly, these exert low immunogenic effects and secrete a variety of cytokines and growth factors. These merits make MSCs favorite candidates to be used in regenerative medicine.[49] In situ transplantation of MSCs to patients, systemic delivery of MSCs, or transplantation of specific cell types obtained after controlled in-vitro differentiation of MSCs is common means of using MSCs therapy to treat diseases in regenerative medicine. [50]

Several studies used MSCs as therapeutic agents to treat cardiovascular diseases, hepatic diseases, lung tissues, renal tissues, and damages to cartilage tissue. A study was conducted to evaluate the effects of MSCs therapy in the myocardial infarction mice model. This study showed that following the systemic delivery of MSCs, these moved towards the myocardial infarction site and homed there. At the damaged site, MSCs differentiated into vascular smooth muscle cells, vascular endothelial cells, and cardio-myocyte, thus repairing the damage and improving the cardiac tissue's function. [51]

Another study reported the therapeutic effects of MSCs therapy in damaged lung tissue. The findings showed that MSCs moved towards damaged lung tissue, differentiated there, and repaired the damage. Furthermore, MSCs secreted the inflammation inhibitory and growth-promoting substances to repair the damaged tissue. [52]

Recently a study investigated the therapeutic effects of insulin-like growth factor-1 containing MSCs in the myocardial infarction mice models. The findings showed that the transformed MSCs reduced the infarction and prevented the cardiac tissue from apoptosis and fibrosis.[53]

In clinical studies, MSCs therapy also proved to be an effective regenerative medicine treatment approach. In 2003, a clinical study was conducted in which six patients with myocardial infarction were given autologous bone marrow-derived MSCs. It improved the functions of left ventricles in four patients and reduced the infarction size in five patients. [54]

Another study reported that the systemic delivery of allogenic MSCs into a patient suffering from severe aplastic anemia promoted the hemopoietic function of bone marrow by releasing hemopoietic growth factors. It resulted in the improved functioning of the bone marrow matrix. [55] Similarly, MSCs therapy-based clinical studies in liver cirrhosis also showed that the transplantation of MSCs or cells obtained from controlled in-vitro differentiation of MSCs resulted in enhanced liver functioning. [56]

Recent studies revealed a variety of MSCs' biological properties and future clinical applications. These findings suggest that MSCs based therapies could be effective regenerative medicine therapy approaches. MSCs will need to be better understood in future research from a multidisciplinary approach to meet the objective of routinely utilizing MSCs in regenerative medicine.

The immunoregulatory function of MSCs in human diseases

Besides the role of MSCs in regenerative medicine, these are also known to modulate immune responses. MSCs can regulate immune effects triggered by autoimmune diseases, tissue damage, and cell transplantation. [57]

MSCs are known to suppress the activation and propagation of B and T cells, growth and differentiation of dendritic cells. These could promote immune tolerance by stimulating the growth of regulatory T cells and suppressing the function of NK cells and macrophages. [58]

MSCs could exert inflammation inhibitory effects; repair damaged tissue and home at inflammatory or damaged tissue sites. Given these merits, MSCs have been utilized in many clinical studies to treat autoimmune

disorders, inflammatory diseases, and graft-versus-host disease (GVHD). In 2008, a preclinical study was conducted to evaluate the effects of MSCs therapy in arthritis. MSCs were given to the collagen-induced arthritis mice model. This treatment increased the population of FoxP3 expressing CD4þCD25þ regulatory T cells, reduced the volume of inguinal lymph nodes, and decreased the swelling of the ankles. Findings showed that MSCs treatment efficiently treated arthritis in mice models. [59]

Acute lung injury is considered a fatal health condition that damages alveolar epithelial and capillary endothelial cells. Studies have reported that MSCs treatment could be beneficial for acute lung injury as it diminishes the alveolar-capillary permeability and extravascular pulmonary edema of lung tissue.[60] Similarly, a treatment approach using umbilical cord-derived MSC was conducted. The findings showed that MSCs treatment prevented lung injury in Escherichia coli mediated sepsis in mice model. [61]

Another study reported the therapeutic success of MSCs therapy in a sepsis mouse model. Following transplantation, MSCs released PGE2, which reprogrammed the functions of macrophages so that macrophages started releasing high levels of IL-10. Thus, anti-inflammatory effects were produced, and mice models were relieved of sepsis.[62] One study evaluated the effects of MSCs treatment in GVDH in the liver and intestine. The results showed that five days following MSCs administration, bilirubin levels decreased in the patient's body. After two weeks, the patient resumed eating activities. [63]

MSCs could regulate the function of T cells. By releasing chemokines like monocyte chemoattractant protein-1 (MCP-1), CXCL9, CXCL10, and CXCL11.[64] MSCs can attract T cells. By releasing cytokines like TGF-b, HGF, IL-10, and PGE2, MSCs can suppress the growth and activation of T cells. By the actions of cytokines, MSCs could also decrease inflammation by increasing the population of regulatory T cells, TH2 and TH17 cells.[65] A phase I clinical study showed that adipose-derived MSCs treatment could be beneficial for inflammatory bowel disease. It reported that fistula healed inpatient after eight weeks of MSCs administration and did not recur within one year.[66]

MSCs also proved to be suitable candidates for treating systemic sclerosis. In a clinical study, a patient was given allogenic MSCs, and the patient's condition started to improve within seven months of treatment. [67] Sun L conducted a clinical study to treat systemic lupus erythematosus (SLE) using MSCs. The treatment strategy showed significant effectiveness against SLE and improved kidney function inpatient after MSCs delivery. [68] Jiang R conducted a clinical study in which 10 patients suffering from type II diabetes were recruited. Patients were administered with placenta-derived MSCs thrice every month for three months. This treatment decreased insulin dependency and increased the levels of the serum-c peptide in treated patients. Further, MSCs therapy also alleviated diabetic-associated renal and CVD complications. [69]

The findings of the studies, as mentioned earlier, revealed that MSCs could provide potent therapeutic effects in the treatment of autoimmune disorders, inflammatory disorders, and GVHD. However, certain inconsistencies are still associated with MSCs therapies, like lack of data about standard transplantation dose, standard delivery method, and standard treatment time. Further research needs to be conducted to solve such issues to get more accurate and potent effects of MSCs therapy.

Treatment of tumors through MSCs therapy

As the tumor grows, the tumor cells release significant quantities of immune mediators, which create an inflammatory state in the tumor microenvironment.[70] The immune mediators secreted by tumor cells could attract MSCs to the tumor site. Many studies investigated the ability of MSCs to migrate towards the tumor site. These studies showed that MSCs could travel to tumor sites in different types of cancers like lung cancer, brain cancer, prostate cancer, colon cancer, pancreatic cancer, skin cancer, and ovarian cancer. MSC also exhibited “homing” at tumor site. [37, 71]

Given the advantages of MSCs like low immunogenicity, tumor homing makes them suitable candidates for developing strategies for cancer therapy. [72]

Tumor therapy with MSCs: the mechanism of action

The employment of MSCs in cancer therapy is gaining popularity among the scientific community. Several studies reported that MSCs could affect tumor cell functioning by decreasing tumor cell viability, inducing cell cycle arrest and apoptosis. MSCs are reported to

have low or minimum effects on normal cells. A study conducted by Lu Y showed that MSCs suppressed the tumor cell growth and spared the normal cells. [73]

Dasari reported that cord blood-derived MSCs attenuated the levels of apoptotic inhibitory protein and suppressed the glioma cell growth. Sasportas also reported that MSCs traveled to the glioma tumor site and induced apoptosis of glioma cells. Another study reported that TNF-a-activated MSCs enhanced the expression of TRAIL (TNF-related apoptosis-inducing ligand) and caused tumor cell death. [74-76]

Moreover, Atsuta reported that MSCs could trigger the binding of Fas ligand (Fas-L) with Fas receptors on tumor cells and could cause cell death in multiple myeloma mice models. MSCs suppressed the tumor growth and inhibited the migration of tumor cells towards the lungs and kidneys. By doing so, MSCs therapy prolonged the mice model survival rate. [77]

Recently, a study reported that umbilical cord-derived MSCs (UC-MSCs) could stop the progression of the cell cycle at G0/G1 phase in leukemia cells by suppressing the expression of oncogenes. Yuan Che reported that exosomal miR-143 isolated from bone marrow-derived MSC could negatively affect the activity of trefoil factor 3 (TFF3) in prostate cancer cells to suppress their migration and invasion. [78, 79]

The above data showed that MSCs could suppress the growth of tumor cells and could inhibit their migration and invasion. Similarly, these can cause apoptosis and cell cycle arrest in different cancer cell types.

MSCs could deliver genes of interest at the tumor site

Almost three decades ago, the idea of utilizing MSCs for gene therapy was proposed by professor Armand Keating. Because of important characteristics of MSCs like tumor homing and evasion of host immune responses, genetically engineered MSCs could exhibit promising anti-tumor effects. MSCs expressing tumor suppressor genes or carrying tumor inhibitory agents to the tumor site have proven effective against cancer. [80, 81]

For the first time, James produced transgenic changes in MSCs and transfected MSCs with the IL-3 gene to make them IL-3 expressing cells. This modification enabled MSCs to express IL-3 without any changes in their innate characteristics. Further, Studeny constructed interferon b (IFN-b) expressing MSCs to treat melanoma and achieved successful tumor inhibitory results. [82] These findings encouraged other researchers to work in this field of research. Xin engineered CX3CL1- overexpressing MSCs and performed systemic delivery of these MSC to lung tumor mice models. Modified MSCs inhibited tumor growth and increased the survival rate of mice models. [83] Seong also conducted systemic delivery of green fluorescent protein (EGFP) labeled TNF-related apoptosis-inducing ligand (TRAIL) expressing MSCs to glioma mice models. Seven days after the delivery, green fluorescence was detected in mice brains, and further monitoring showed that tumor growth reduced significantly after 14 and 21 days. The results indicated that MSCs migrated to the brain and inhibited tumor growth.[84] Qiao manufactured MSCs containing osteoprotegerin gene expressing adenoviruses and injected them in osteoma mice models. The findings showed that the treatment remained successful, and osteoma growth was inhibited. [85]

Our research group engineered E1s modified MSCs to carry replication-deficient adenoviral vector p53/p21 expressing MSCs to the tumor site. In mice models, the novel MSCs based delivery was employed to carry therapeutic adenoviral vectors to prostate and lung tumor sites. Results showed that MSCs containing adenoviral vectors reached the tumor site, home there, and inhibited the growth of tumors. [86, 87]

The above data showed that MSCs could be employed to deliver the gene of interest to tumor site in an efficient manner.

MSCs could carry drugs at the tumor site

Besides gene delivery, MSCs also showed promising potential to deliver drugs to the tumor site. MSCs could carry drugs, move towards the tumor site and deliver the drug to the tumor site. Nanoparticles are the most prominent drug coating materials that can be packaged into MSCs and delivered to tumor sites. Drug coated polymer and lipid nanoparticles can be packaged into MSCs by endocytosis and lipid fusion, respectively. [88, 89]

Data showed that within seven days of packaging of nanoparticles into MSCs, the innate characteristics of MSCs like the survival, differentiation, and tumor-homing remained unaffected. [90] A study investigated the percent survival of nanoparticles in MSCs. The result showed that three days after the packaging of MSCs

with polymer nanoparticles and lipid nanoparticles, 95% of cells showed the presence of polymer nanoparticles, and 45% of cells showed the presence of lipid nanoparticles. The reason for a decrease in the percentage of the nanoparticles containing cells is thought to be cell division.[91]

MSCs deliver the drug-coated nanoparticles to the tumor site possibly through the following mechanism: Nanoparticles enter the MSCs in the form of endosomes. After entry, these are engulfed by lysosomes. The nanoparticles remained unaffected by the actions of hydrolytic enzymes in lysosomes. The nanoparticles disrupt the lysosomal membrane and release into the cytoplasm. Further, these nanoparticles enter into tumor cells, thus, deliver the drug to tumor cells. [92, 93] Several studies have demonstrated the therapeutic effects of MSCs containing drug-coated nanoparticles. MSCs have been utilized to deliver coumarin-6 and silica nanodoxorubicin drugs to the tumor site to treat glioma. [94] Moreover, MSCs have also been used to deliver porphyrins-coated fluorescence core-shell nanoparticles and paclitaxel-coated nanoparticles to treat osteoma and lung cancer, respectively. [95, 96] The above findings show that MSCs could be promising carriers of drug-coated nanoparticles.

Some studies have highlighted the controversial role of MSCs in tumor treatment. Although MSCs proved to be promising anticancer agents that could act directly as antitumor agents or carry antitumor agents to the tumor site. It has been reported that MSCs could promote tumor growth and angiogenesis. [97] So, employing MSCs in clinical settings requires more data on safety, effectiveness, and a better understanding of mechanisms responsible for MSCs and tumor cell interaction.

The applications of MSCs in clinical studies

For the last two decades, MSCs have been extensively utilized in clinical settings to evaluate the efficiency of MSCs based cell therapy in various health conditions. Many clinical trials are being conducted to investigate the effectiveness of MSCs therapy in several human disorders. [98, 99]

MSCs possess particular abilities, including the ability to home at the site of inflammation or tumor, low immunogenicity, viral vectors or drug load carrying capacity, immune regulatory capacity, the release of various chemical substances, multipotent differentiation. These merits make MSCs the suitable candidates to be used in clinical studies.[100]

Literature review showed that the first MSC-based clinical study was conducted in 1995 in blood cancer patients. [101] In this study, MSCs were isolated from patients, isolated MSCs were grown in-vitro, and then patients received autologous MSCs transfusion. Further, another clinical study employed allogenic MSCs transfusion to patients with GVHD and obtained promising therapeutic results. [102] Optimistic Results of many MSCs therapy-based clinical studies encouraged researchers to investigate MSC therapy's efficacy in various health conditions. Public Clinical Trial Database shows that 3,779 MSCs based clinical studies are being conducted to check the therapeutic effects of MSCs therapy in a wide range of pathological conditions.

The data shows that most clinical trials are in phase 1 or II, and fewer clinical studies have entered in phase II or IV. Besides ongoing studies, participant recruitment for new clinical studies is also going on (http://clinicaltrials.gov) as of 20th October, 2021.

Several clinical studies conducted to treat various human diseases like acute myocardial ischemia, stroke, cirrhosis, amyotrophic lateral sclerosis, and GVHD have been completed. The findings of these studies showed that MSCs based therapies produced safe and potent therapeutic effects in patients.

The limitations of MSCs therapy in clinical studies

Although MSCs-based therapies proved to be potent and safe in many clinical trials. Some recent studies showed that MSCs could pose potential risks during therapy. MSCs could undergo misdifferentiation, these could promote tumor growth and could induce unwanted immunosuppression. [103]

A study reported misdifferentiation of MSCs following transfusion in glomerulonephritis mice models. After reaching renal tubules, MSCs differentiated into adipocytes, interfered with normal kidney functions, and promoted chronic kidney diseases. [104] It is also observed that MSCs could form microemboli in the capillaries of mice models and contribute to forming osteosarcoma-like lung lesions. [105] Additionally, it is also found that MSCs could induce unwanted immune

suppression. In a clinical study, MSCs were given to GVDH patients. Following treatment, 1/3 of patients showed viral infection. The immunosuppressive effects of MSCs were thought to be the reason for viral infection in the patients.[106] MSCs are also found out to be associated with the promotion of tumor growth. Unmodified MSCs could differentiate into tumor cells. These could minimize the effects of drugs by metabolizing the chemotherapy drugs and also could attenuate the host anti-tumor immune response.[107]

Several studies have reported that under certain conditions, MSCs could transform in tumor cells. [108] Similarly, it is found that MSCs metabolized asparaginase drug in acute lymphoblastic leukemia cells. By doing so, these greatly minimized the effects of chemotherapy in acute lymphoblastic leukemia.[109]

The above data showed that great care should be taken in the selection of patients for MSCs therapy. Patients with poor immune conditions and patients with chances of developing tumors must be carefully given MSCs therapy. Furthermore, there is a need to gather more data on the safety and effectiveness of MSCs based therapy, especially in clinical settings.

Conclusions

MSCs considered the most important stem cells among adult stem cells. MSCs can be obtained from a wide range of sources. MSCs could undergo self-renewal and differentiate into multiple cell types. MSCs have the ability to move towards injured, inflammatory, and tumor sites. These could reach the sites of regeneration and could home there with minimal host immune activation. MSCs could also act as carriers of viral vectors and drugs. These can selectively carry the therapeutic agents to a target site in the body. Given these merits, MSCs have been extensively investigated in preclinical and clinical studies for their therapeutic effects. MSs have shown potent therapeutic effects in cancer, autoimmune diseases and also successfully repaired various damaged tissues. Several MSCs therapy-based clinical studies at different levels are being conducted to treat a variety of human diseases. Some studies also reported few demerits associated with MSCs therapy in preclinical and clinical settings, which urges the careful selection of MSCs therapy for different patients. Because of all these findings reported in the literature, we have summarized the use of MSCs therapy in important health conditions, including cancer, autoimmune diseases, and tissue regeneration. The information about the application of MSCs in therapeutic strategies may be beneficial in the better understanding of pros and cons of MSCS therapy in different human diseases.

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Open Access Original Article

Analysis of Discard Rates of Blood and Blood Components as A Quality Indicator to Improve the Blood Transfusion Services of a Tertiary Care Hospital in South India

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Abstract

Objective: Blood is recognized as a precious resource gifted by the blood donors to patients in need. This study was taken to understand the reasons for discard of donated blood units.

Methodology: The discard rate of blood and blood components was quantified as wastage as percentage of issue (WAPI) using last five years records. This was a retrospective study done using the past five years (2015-2019) records available with the Transfusion Medicine department of Sree Chitra Tirunal Institute for Medical Sciences and Technology.

Results: Our overall wastage as percent of issue (WAPI) over the last five years was 6.14%. WAPI for Packed red cells, Fresh frozen plasma and platelets was 4.23%, 3.56%, and 16.6% respectively.

Conclusions. Even though wastage rates were comparable with published literature from other parts of the country, this study helped identify areas of intervention to further minimise the wastage.

Keywords: Blood component, blood transfusion service, discard rate, fresh frozen plasma, quality indicator.

Introduction

Blood is a precious resource gifted by donors for the patients. World Health Organisation (WHO) has included blood in its list of essential medicines[1] to highlight the life-saving role of blood. The demand for blood is still very high and the supply is below the demand levels.

According to the latest annual report published by the Ministry of Health and Family Welfare, India, the annual requirement for blood in India is estimated at 13 million units based on the WHO norm of 1% of the population, and our collection was 12.5 million units.[2] Therefore, each unit of blood should be optimally and judiciously utilized.

The AABB defines quality indicators as the specific performance measurements designed to monitor one or more processes during a defined time and are useful for evaluating service demands, production, adequacy of personnel, inventory control, and process stability of the blood transfusion services (BTS).[3] One such quality indicator for assessing the performance of BTS is the discard rate of blood and blood components.

The discard rate of blood and blood components is quantified as wastage as percentage of issue (WAPI). In an ideal situation, the discarding and wastage of preciously donated blood and blood components should never occur. The discard rates of blood and blood components reflect upon the overall planning and technical efficiency of the department and its coordination with other clinical departments utilizing the transfusion services.

By analyzing the data and the reason for the discards, the blood transfusion service can develop plans to improve performance through education and training of staff and introduce new measures in order to minimize the number of discarded blood units to the minimum possible. The study was undertaken with the

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following objectives. 1) Analyse the discard rates of blood and blood components in our BTS, 2). Analyze the various reasons for discard of blood and blood components & Suggest steps to minimize the number of discarded blood units and improve the overall efficiency of BTS.

Methodology

This was a retrospective study done using the past five years' (2015-2019) records available with the Transfusion Medicine department of Sree Chitra Tirunal Institute for Medical Sciences and Technology. Institutional ethics committee clearance was obtained prior to the start of the study (SCT/IEC/1640/Dec-2020).

Statistical Analyses: Data relating to the collection and usage of blood and its components from Jan 2015 to Dec 2019, were obtained from the records of the Transfusion Medicine department. The reasons for the discard of units during this period was tabulated and analysis done. The analysis of the reasons for discard of blood units were broadly classified into donation, processing, storage, and post issue related, to determine the type of intervention required to minimize the wastage. Table I lists the reasons for discard under each category.

Table I: Categorization of reasons for discard of blood units

Donation related	Processing related	Storage related	Post issue
Under/Overcollection	Bag broken/leaking during centrifugation	Bag broken during storage/thawing	Unused after issue
Confidential unit exclusion (cue)	Cell contamination	Storage temperature not appropriate	Bag leak
Lipemia	Low volume	Expiry of shelf life	Precipitates
Antibody positive	Hemolysis during leucofiltration	Hemolysis	Cold chain not maintained
Presence of clot		Absent swirling	
TTI reactive status			

Data were used to calculate:

1) Wastage rate = Number of blood and blood components discarded/ Total number of blood and blood components issued x100

2) Percentage wastage for each reason = Number of units discarded for each reason/ Total of number units discarded for all reasons

Results

The total number of blood units collected in the five years 2015-19 together was 36,280. Of these, 304 units were discarded as whole blood. The commonest reason for discard as whole blood was insufficient volume collection (87.82%) either due to poor venous access (43.2%) or due to development of vasovagal reactions during collection (56.8%). Thirteen units (4%) were discarded due to broken/leaking bag during the processing. Eight units (2%) were discarded as confidential unit exclusion.

About 0.83% of the units had to be discarded as whole blood due to low volume collection either due to poor venous access or development of vasovagal reactions during donation. Cumulative WAPI for five years was 6.14%, which is lower than other published studies from different parts of India (Table II)

A total of 78,302 blood components were issued for transfusion during the five years, which included 48.12% packed red cells (PRC), 38.5% Plasma (including FFP and cryoprecipitate) and 13.38% platelets. 4318 units (5.51% of total issue) were discarded, of which 36.51% were PRC, 23.45% were plasma and 40.04% were platelets. Analysis of the trends in total number of blood units collected, components prepared, components issued and discarded during the preceding five years shows an

Table II: Comparison of present wastage data of blood units with published literature.

	PRC (%)	Plasma (%)	Platelets (%)	Overall discard (%)
M Morish et al[4] (Malaysia, 2012)	4.1%	4.5%	6%	2.3%
H J Shahshahani et al[5] (Iran, 2017)	2.9%	2.3%	10.5%	2.1%
Sadia Sultan et al[6] (Pakistan 2021)	0.7%	0.009%	4.5%	10.9%
Ryan A. Collins et al[7] (2015, US)	0.56%	2.81%	1.40%	1.59%
Kurup R et al[8] (2016, Guyana)	Not available	Not available	Not available	25.4%
S A Kafi-Abad et al[9] (2019, Iran)	5.7 ± 0.7%	1.4 ± 0.4%	3.2 ± 0.5%	3.4%
Kaur et al[10] (2015, North India)	6.86	7.96	56.31	10.29
Simon et al[11] (2020, South India)	3.5	5.5	52%	19.3%
Kanani et al[12] (2017, West India)	2.26	5.36	28.39	6.95
Suresh et al[13] (2015, South India)	3.8	5.5	16.6	7
Kumari et al[14] (2019, East India)	21.4	11.7	66.4	22.8
Present study (2021, South India)	**4.23**	**3.56**	**16.6**	**6.14**

increase in the number of blood unit prepared and issued in 2018, as compared to preceding and succeeding years, even though collection showed only a modest increase (Figure 1). This was due to increased production and utilisation of platelets. The discard rate has remained steady in the past five years.

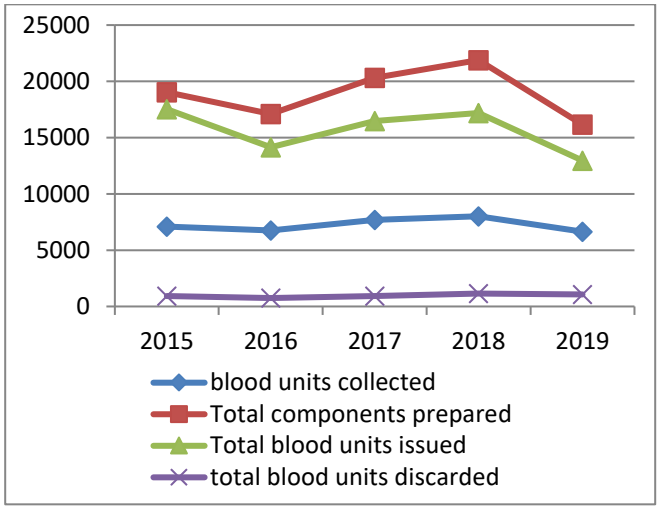
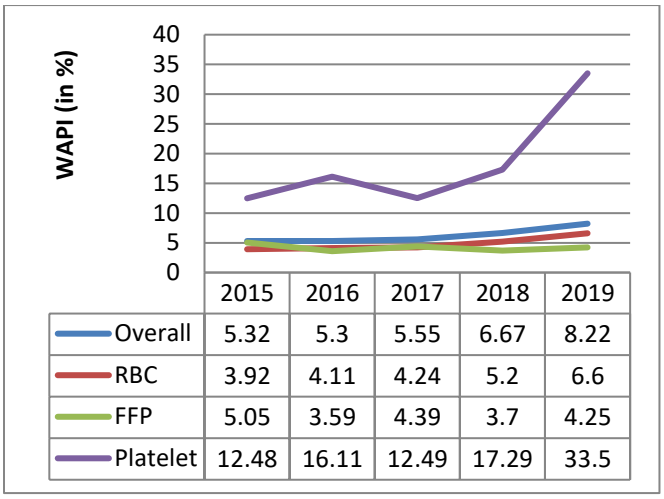


Figure 1. Trends in collection, preparation, issue and discard of blood components.

Yearly wastage as percentage of issue (WAPI) for all blood components together was 5.32, 5.30, 5.55, 6.67 and 8.22% respectively during the five years from 2015-19 and five years cumulative WAPI was 6.14%. Cumulative WAPI for packed red cells, fresh frozen plasma, and platelets was 4.23%, 3.56%, and 16.6% respectively. Figure 2 illustrates the trend of WAPI for the different blood components during the five years.

The reasons for blood unit discard were broadly categorised based on reasons listed out in table 1. Storage related (Expiry of shelf life of units) was the commonest cause of discard for PRC and platelets followed by a donation related (reactive status on transfusion transmitted infection (TTI) testing). For plasma components, discard was most commonly due to donation related, followed by storage related (Figure 3).



	2015	2016	2017	2018	2019
Overall	5.32	5.3	5.55	6.67	8.22
RBC	3.92	4.11	4.24	5.2	6.6
FFP	5.05	3.59	4.39	3.7	4.25
Platelet	12.48	16.11	12.49	17.29	33.5

Figure 2. Trends in the WAPI rates between 2015-19

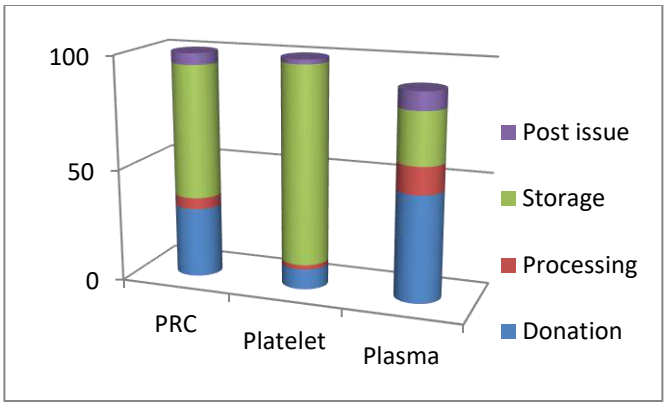


Figure 3: Analysis of discard causes category wise

About 53% of PRC was discarded due to expiry of shelf life, and 32% due to reactive status on TTI testing. The rates were 85% and 9% for platelets. For plasma components, 42% discard was due to reactive status on TTI testing, followed by bag breakage in 19%. (Figure 4)

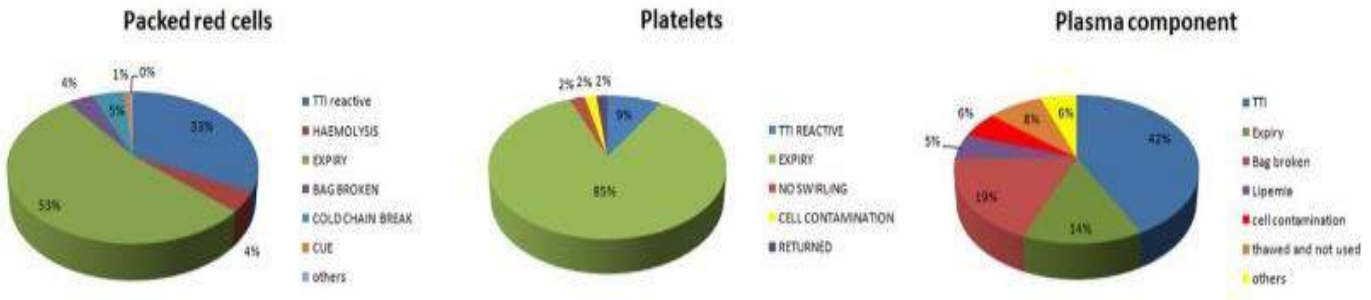


Figure 4: Reasons for discard of Blood components

Discussion

Hematopoietic stem cell transplantation (HSCT) is an Platelets were among the majority of all discarded blood components. This is similar to all other published studies except one study from north India, where the majority of donated units were discarded as whole blood (Table III). Analyzing the reasons for discard, expiry of shelf life (particularly platelets) was the commonest cause; this is due to the fact that most of our blood requests are for PRC for intra-operative use. Platelets use is restricted to cases of massive transfusion and emergency surgeries, where the patient is on dual anti-platelets. Hence, we need to keep a reserve of platelets available for such unexpected needs, which gets discarded if not used within the short shelf life. Plasma is used mainly in cases of therapeutic plasma exchange and massive transfusions.

Of the discarded blood components, 50.88% was due to expiry of shelf life, and 22.51% were due to TTI reactive status. 53% of the PRC was discarded due to shelf life expiration, and 32% due to reactive status on TTI testing. For platelets, 85% was due to expiry and 9% was due to TTI reactive status. For plasma components 42% discard was due to reactive status on TTI testing, followed by bag breakage in 19%.

During these five years, various steps were taken to minimize the wastage rates. We switched to 100% voluntary blood donation to increase blood safety and discard due to TTI reactivity.[15] PRC with additive solution was introduced to increase the shelf life. Excess plasma components were given for fractionation. Studies were initiated to understand the frequency and aetiology of massive transfusions to better prepare the inventory. Emphasis on implementing massive transfusion protocols with optimum use of platelets and plasma components was highlighted in the meetings of hospital transfusion committee.[16]

Conclusion

Donors, staff at blood centre, and end users of blood units are equal stakeholders to ensure an efficient blood transfusion service. Blood is a valuable resource, and wastage of blood units has a negative impact on BTS. This study helped in identifying more areas for intervention to further reduce the discard rates.

1) Donation related – timing between last food intake and donation to minimise discard due to lipemia, ensuring good venous access before venepuncture to minimize low volume collection

2) Processing related- Identify the cause for hemolysis in each PRC units (like temperature dysregulation, leucofiltration, high haematocrit etc.), training and supervision in proper loading of blood bags in centrifuge cups to prevent dent marks

3) Storage related -Networking with other neighbouring blood banks to supply the near expiry blood components to minimize discard due to expiry of shelf life, strictly following first in first out policy in case of massive transfusions, use of individual stands to store FFP to prevent sticking and bag breakage, regular checking of thermographs and temperature alarms in all blood storage equipment

4) Post issue – sensitize the clinical staff regarding rational blood use, ordering according to existing

Table III: Comparison of discard rate and reasons for discard of blood units from published literature

	Total discard	Cause -1	Cause-2	Cause-3	Most discarded
M Morish et al[4]. (Malaysia, 2012)	2.3%	Red cell contamination	leakage	lipemia	Platelets
H J Shahshahani et al[5](Iran, 2017)	2.1%	Expiry	Processing related	Storage related	Platelets
Sadia Sultan et al[6](Pakistan 2021)	10.3%	TTI reactive	expiry	Red cell contamination	Platelets
Ryan A. Collins et al[7](2015, US)	1.59%	Expiry	Storage related	Returned >30 min	Platelets
Kurup R et al[8](2016, Guyana)	25.4%	Expiry	Broken seal	Broken cold chain	Platelets
S A Kafi-Abad et al[9](2019, Iran)	3.4%	Expiry	Reserved/returned units	Bag breakage	Red blood cells
Kaur et al[10](2015,North India)	5.8%	Expiry	TTI reactive	Bag breakage	Whole blood
Simon et al[11] (2020,South India)	19.3%	Expiry	TTI reactive	Bag breakage	Platelet
Kanani et al[12](2017,West India)	6.95%	Expiry	Low Volume	Bag breakage	Platelet
Suresh et al[13](2015,South India)	7%	TTI reactive	Low volume	Quality control	Platelet
Kumari et al[14] (2019, East India)	22.8%	Expiry	TTI reactive	Bag breakage	Platelets
Present Study(2021,South India)	6.14%	Expiry	TTI reactive	Bag breakage	Platelet

maximum surgical blood order schedule, over the group compatible platelet use, maintenance of cold chain after issue, prompt return of unused units, transfusion audits to ensure good transfusion practices.

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Open Access Original Article

Clinico-Hematological Characterization of Pakistani Patients with Fanconi Anemia

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Abstract

Objective: This study was designed to characterize the clinical and hematological aspects of Pakistani Fanconi anemia (FA) patients.

Methodology: The study was conducted in Armed forces institute of Pathology Rawalpindi and University of Health Sciences Lahore from 2012 to 2016. Seventy unrelated patients with bone marrow failure were included in this study. Forty FA patients diagnosed by chromosomal breakage analysis were assessed for their demographic, clinical and laboratory parameters. SPSS version 20 was used for statistical analysis.

Results: This study showed mean age of seeking medical aid as 11.21 ±4.22 years. Prenatal consanguinity was 65% and 16 out of 40 patients had siblings affected with FA. All of them had history of repeated infections. There was pleomorphic presentation and varying degree of pancytopenia. Short stature, microcephaly, microphthalmia and café ul lait spots were the common findings.

Conclusion: Fanconi anemia is genetically a heterogenous disease with wide variability of phenotypic presentation. Knowledge of pleomorphic clinical presentation may support health professionals in early diagnosis, evaluation of prognosis, monitoring and genetic counselling of patients and families with Fanconi anemia.

Key words: Fanconi anemia, Inherited, autosomal recessive, Bone marrow failure, Pakistani Consanguinity.

Introduction

Fanconi anemia (FA) is a rare genetically heterogenous disease that was first described by a Swiss Pediatrician Guido Fanconi in 1927. Mode of inheritance in most of the cases is autosomal recessive whereas rarely it is inherited in X-linked pattern (FANCB) and autosomal dominant (FANCR) manner of inheritance has also been described.[1]

The incidence of FA is 1 in 160,000 to 360,000 live births. Though the carrier frequency in general population is 1:300, it is higher in certain ethnic groups such as South African Afrikaners, Spanish Gypsies and Blacks of Sub Saharan. Male and females are equally affected with clinical presentation of bone marrow failure manifestations at median age of 8 years.[2]

Fanconi anemia is characterized by Congenital anomalies, progressive bone marrow failure and early onset malignancies. The most common physical abnormalities include short stature, hyper or hypo pigmentation of skin, Café-au-lait spots, radial ray abnormalities, central nervous system, gastrointestinal, cardiovascular and renal malformations. Progressive bone marrow failure reveals mostly in first decade of life with thrombocytopenia, leukopenia and anemia. Macrocytosis may be the first detected hematological abnormality. Along with these FA patients are predisposed to Acute myeloid leukemia and squamous cell carcinoma.[3,4]

Maintenance of genomic stability depends on specialized mechanisms in the body that serve to repair DNA damage, caused by exogenous or endogenous sources. Hypersensitivity to Interstrand cross linking agents (cisplatin and mitomycin C etc.) and inability of interstrand crosslinks (ICLs) repair is the hallmark feature of Fanconi anemia diagnosis.[5]

Twenty-two FA genes have been identified till date. They include FANCA, FANCB, FANCC, FANCE, FANCF, FANCG (XRCC9), FANCL, FANCM, FANCT (UBET2), FANCP (SLX4), FANCD2, FANCI, FANCD1

Authorship Contribution: [1]Conceived and planned the idea of the study, final approval of the version to be published, Collecting the data, [2]drafting the work or revising it critically for important intellectual content, [3,4]Data Analysis, literature review.

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(BRCA2), FANCJ(BRIP1), FANCN (PALB2), FANCO (RADS1C), FANCR (RADS1), FANCS (BRCA1), FANCU(FRCC2), FANCV (REV7/MAD2L2), FANCW (RFWD3) and FANCQ (ERCC4, XPF). The FANC proteins along with associated proteins repair the interstrand crosslinks (ICLs) formed during S phase of the cell cycle by a pathway known as FA pathway of FA/BRCA pathway. Mutation in any of the FA protein impairs the repair mechanism leading to development of disease.[6]

Fanconi anemia is the most common inherited bone marrow failure syndrome. Limited studies and research subjected this disease disavow particularly its prevalence in highly intermingled and consanguineous cultural back ground of Pakistan. In a study conducted by wali et al in 2011 revealed the frequency of FA as 16.6% among 90 aplastic anemia patients.[7] This signifies the importance of FA registry in Pakistan; early diagnosis, timely management and avoidance of complications. FA is phenotypically a heterogenous disease with variable severity. Current study presents the demographic, phenotypic and laboratory parameters of Pakistani FA patients.

Methodology

The study was approved by institutional review committee University of Health Sciences Lahore. Declaration of Helsinki Written, informed consent was obtained from all patients or parents. Study was conducted in n Armed forces institute of Pathology Rawalpindi and University of Health Sciences Lahore.

In this study, we initially included 70 patients with Bone marrow failure from 2012 to 2016 who presented in Armed Forces Institute of pathology Rawalpindi and Children Hospital Lahore. Recruitment of patients was based on detailed history, general Physical and systemic examination and hematological findings of bone marrow failure (BMF). BMF was considered as decrease in one or more blood cell lines in complete blood count, reduced hematopoiesis seen in bone marrow aspiration smears attained with Giemsa stain and hematoxylin-eosin-stained bone marrow trephine biopsies according to the World health organization classification of tumors of hematopoietic and lymphoid tissues 2008. Data was collected and noted in designed proforma. Final diagnosis of Fanconi anemia in these patients was made by positive chromosomal breakage assay i.e., Mitomycin C (MMC) test.

Chromosomal breakage assay

DEB/MMC test was performed according to the protocol described earlier.[8] It was performed by inoculating peripheral venous blood in RPMI 1640 and Fetal bovine serum (FBS) and phytohemagglutinin (PHA). Cultures were kept in Oxygen incubator for 72 hours at 37^{o}C. After 24 hours of inoculation, cultures were induced with Mitomycin C. For each culture a replicate tube was paired as untreated control. Colchicine was added to arrest the cultures at metaphase stage and then treated with Potassium chloride solution (10%). The cells were prefixed with methanol glacial acetic acid solution. Slides were stained with Giemsa stain. Twenty to twenty-five metaphases on at least 4 slides for each patient were seen and scored under bright field microscope and compared with negative controls.

Forty patients with positive DEB/MMC test were recruited after confirmed diagnosis of Fanconi anemia. Patients of any age and both genders were assessed for their demographic, clinical and laboratory parameters.

Results

In this study 40 patients of Fanconi anemia (19M:21F) were included after informed consent. Mean age of patients was 11.21 ±4.22 years ranging from 5 years to 20 years. Weight and height were 10-60 kg (mean 28.00±13.07 kg) and 35-63 inches (mean 47.65±7.18 inches) respectively. Parents of 26 (65%) patients had consanguineous marriage whereas of 14 (35%) patients had no history of consanguinity and were totally unrelated before marriage (Figure 1).

The siblings of 16 patients had the same disease. Bruises or epistaxis found to be the first symptoms and majority of patients seeked medical attention due to recurrent infections with mean age at the time of presentations as 7.95 ± 4.56 years ranging from the age of one month to 19 years. The most common finding in these patients was recurrent infections (100%) followed by pallor (90%), Bruises (72.5%) and epistaxis (52.5%) Thirty-three (82.5%) patients had one or more congenital anomalies whereas 7 (17.5%) patients had no congenital anomaly (Table I)

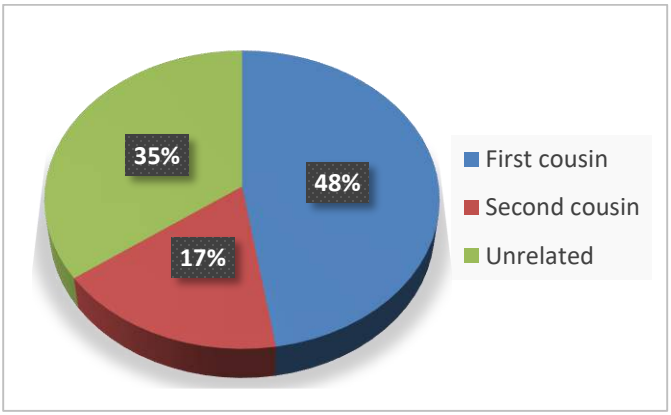

Figure-1: Frequency of prenatal consanguinity among Pakistani Fanconi anemia Patients

Table I: Clinical characteristics of Fanconi anemia patients

Variable		Frequency	%
Gender	**Male**	**19**	**47.5**
	Female	**21**	**52.5**
Consanguinity		26	65
Siblings affected		16	40
Pallor		36	90
Bruises		29	72.5
Epistaxis		21	52.5
Recurrent infections		40	100
Low weight		32	80
Short stature		31	77.5
Microcephaly		25	62.5
Microphthalmia		25	62.5
Café ul lait spots		11	27.5
Thumb abnormalities		12	30
Absent/ectopic kidney		4	10
Cardiac abnormalities		3	7.5
Genital tract abnormalities		2	5

Hematological findings of these patients revealed variable degree of cytopenia with mean Hb 8.04±2.57 g/dl, mean White blood cells 7.40± 27.21 x10^9/L and mean platelet count 57.66±43.26 x10^9/L. All the patients had hypocellular marrow for age. All the patients had normal serum electrolytes and 3 out of 40 FA patients had raised liver enzymes. Figure 2 shows the physical abnormalities in patients of our study.

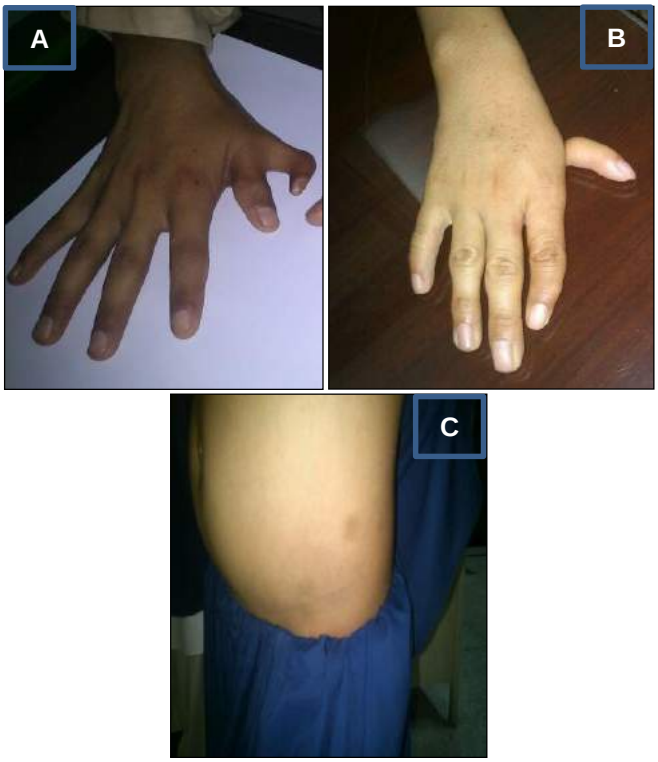

Figure-1: Physical abnormalities in patients with Fanconi anemia of this study. A) Bifid thumb, B) Rudimentary Thumb, C) Café ul lait spots

Discussion

Fanconi anemia is genotypically and phenotypically a pleomorphic inherited disease. It affects all ethnic groups with frequency of 1:360,000 births.[9] Progressive bone marrow failure, high risk of malignancies at an early age and developmental abnormalities are characteristic features. Susceptibility to spontaneous chromosomal breakage is the hall mark of FA cells and chromosomal breakage analysis is the gold standard diagnostic test. Fanconi anemia proteins play a pivotal role in DNA repair mechanism with their associated proteins. Disruption in this repair mechanism by mutated FA proteins leads to Fanconi anemia.[10]

In this study we present a cohort of 40 Fanconi anemia patients. Most of them belonged to Punjab 67.5% (N=27) and rest from Khyber Pakhtunkhwa 32.5% (N=13). High degree of consanguinity was observed. A study conducted in Israel showed 63% consanguinity resulting in this autosomal recessive disorder whereas consanguineous marriages at high level of 94.2 % were responsible for this disorder in a study conducted in Karachi Pakistan.[11,12] Our study showed that 65% patients were offspring of

consanguineous marriages. The results indicate that incidence of Fanconi anemia is higher in couples with family/cousin marriages. Similar results were seen in a Turkish study.[13]

Fanconi anemia is not only a complex disease, but its clinical heterogeneity and overlapping of clinical features with other diseases make it difficult to be diagnosed. Though the disease is associated with the presence of congenital abnormalities, many patients do not have any such abnormalities at the time of birth.[14] Previously, a study conducted by Chowdhry et al revealed common clinical features in 65.8 % of patients, whereas in 34.20% patients there were no apparent symptoms.[15] The most common clinical findings in our patients include short stature, microcephaly, café au lait spots and thumb abnormalities. All patients with congenital anomalies account for 82.55 of those found to have at least one abnormality, 17.5% did not have apparent skeletal deformities. In a previous study done in Mumbai India short stature 81.1 % (N=27) was the most common finding among FA Patients followed by Skeletal abnormalities and skin pigmentation 48.5% (N=16) and 45.5% (N=15) respectively.[16] In our study short stature was the most common finding, and out of 40 FA patients, 77.5% had short stature, followed by microcephaly and microphthalmia each 625% and skin pigmentation in 275% patients. The clinical presentation varies from patient to patient and not all the FA patients have congenital anomalies which signifies the importance of chromosomal breakage analysis in all patients with aplastic anemia for intime diagnosis and early management and diseases associated complications may be avoided.

According to the literature the hematological abnormalities appear at the median age of 7 years.[17] The median age at the time of presentation in our study is 8 years with mean duration of illness as 3.8 years and thrombocytopenia being the first hematological abnormality with spontaneous bruises and epistaxis followed by anemia and finally pancytopenia.

Conclusion

The present study provides the clinical, hematological and biochemical findings in Pakistani Fanconi anemia patients. Our data provided wide phenotypic presentation of FA patients. This heterogenous and multisystemic presentation can offer suspicion to clinicians for timely testing and early diagnosis.

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Open Access Original Article

Seroprevalence of IgG Antibodies Against Severe Acute Respiratory Syndrome Coronavirus 2 and Associated Risk Factors in Blood Donors

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Abstract

Objective: To determine the seroprevalence of SARS-CoV-2 immunoglobulin G (IgG) antibody and identify risk factors among blood donors.

Methodology: This cross-sectional study was conducted at the Armed Forces Institute of transfusion (AFIT), from July to October, 2020. A total of 900 blood donors were enrolled according to the calculated sample size. Sociodemographic, behavioral and clinical data were collected through a preformed questionnaire. Seroprevalence of SARS-CoV-2 IgG antibody was detected using indirect enzyme linked immunosorbent assay (ELISA) of Vircell COVID 19.

Results: Out of a total 900 donors, 180 (20%) were found out to be positive for SARS- CoV-2 antibodies. Of these 180 SARS-CoV-2 IgG positive cases, asymptomatic donors were 140 (77.7%) whereas 60 (33.7%) were symptomatic. Respiratory symptoms were most common and gastrointestinal were least common among seropositive donors respectively. None of the ELISA IgG negative samples were found to be positive for SARS-CoV-2 RNA by RT-PCR.

Conclusion: A high seroprevalence of SARS-CoV-2 IgG antibody reflected a great extent of spread virus in blood donor population.

Keywords: Blood donors, COVID-19 IgG antibody, ELISA, SARS-CoV-2

Introduction

Coronavirus disease 2019 (COVID-19) is caused by a novel beta-coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which was first detected in Wuhan, China at the end of 2019. SARS-CoV-2 infection is manifested with mild or moderate symptoms (dry cough, fever, body aches, and diarrhea) but in a minority of cases, severe symptoms (respiratory distress, pneumonia and cytokine release syndrome) appear, which can be fatal. SARS-CoV-2, a positive sense-single stranded RNA virus, consists of four major structural proteins: Spike(S), nucleocapsid (N), membrane (M) and envelope (E). Rapid transmission of this virus across the globe lead to a global pandemic in 2020.[1,2,3] In Pakistan, SARS-CoV-2 cases were first reported in February, 2020 in individuals who had returned from Iran.4 A substantial burden on fragmented health care systems led to a limited COVID-19 testing mainly in symptomatic cases, thereby leaving asymptomatic cases, which was a source of spread of infection in the general population. [5]

It is significant to determine the seroprevalence of SARS-COV-2 antibody among blood donors to identify the extent of exposure to the disease and the development of possible herd immunity. Detection of antibodies among apparently healthy blood donors gives important information about the circulation of virus as blood donors represent a part of general population. This data can also be used to assess the effectiveness of potential mitigating strategies.[6] SARS-CoV-2 diagnosis is carried out by real time reverse transcription polymerase chain reaction (RT-PCR) but factors such as inadequate sample collection, fluctuating viral load and time between sample collection and symptoms onset influence its sensitivity by giving false negative results.[7] Validated serological assays can facilitate the prompt identification of cases that have been infected with SARS-CoV-2 thereby assisting in enhancing the strategies for blood safety.[8] A previous study conducted

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on blood donors in southern region of Pakistan from May to July, 2020 reported a 40% seroprevalence rate.

During that period, the number of confirmed cases were 0.2 million with 6000 deaths.[5] To date, Pakistan has reported more than 0.4 million cases with 9330 deaths.[9] Considering a surge in SARS-CoV-2 infected patients, this study was conducted to determine the seroprevalence of SARS-CoV-2 IgG antibody and associated risk factors among blood donors. Furthermore, nucleic acid testing was done to detect possible SARS-CoV-2 infection in blood donors.

Methodology

A cross-sectional study based on seropositivity for SARS-CoV-2 was carried out on blood donors from July to October, 2020. All potential blood donors reporting to the Armed Forces Institute of Transfusion (AFIT) during the pandemic period were recruited using convenient sampling technique. The study protocol was approved by an Institutional Ethics Committee (AFIT-ERC-20-010) and written informed consent was obtained from all study participants. Sample size calculation was performed using single proportion formula with 95% confidence interval, margin of error 5% and a prevalence of 37.8% reported in a previous study.[5] The study enrolled 900 blood donors who met the blood donor selection criteria according to World Health Organization (WHO) guidelines. In addition, those donors who experienced any COVID-19 related symptoms[10] in the past 28 days of donation and had contact with confirmed or suspected SARS-CoV-2 patient were excluded.[11] Blood donors who have been previously symptomatic but were free of symptoms for more than 28 days were included. In the study a standardized questionnaire was used to collect sociodemographic data; age, gender and educational level, history of SARS-CoV-2 symptoms such as respiratory, gastrointestinal and behavioral factors like history of smoking. Serological detection of SARS-CoV-2 IgG antibody against recombinant antigen (a mixture of nucleocapsid N and spike protein S) was performed by indirect enzyme linked immunosorbent assay (ELISA) (Vircell Microbiologist, Spain) following manufacturer's instructions using serum used for routine transfusion transmissible infection markers. Internal positive, negative and cut off controls were run with each batch. A serum sample from recovered case with positive IgG antibody status was also included as an additional positive control. ELISA IgG negative samples were tested for SARS-CoV-2 ribonucleic acid (RNA) by real time PCR on Roche Cobas 6800 system. The primary outcome was to assess the seroprevalence of IgG antibody among blood donors while secondary outcome was to determine the association between IgG seropositivity and socio-demographics (age and educational level), behavioral characteristic including smoking and symptoms consistent with SARS-CoV-2 infection.

Statistical package for social sciences (SPSS) version 23.0 was used for data analysis. Categorical variables were presented as mean (SD) and continuous variables as frequency and percentages. Pearson Chi-square test was used to find the association between variables. A $P<0.05$ was considered as significant.

Results

A total of 900 blood donors were recruited during the study period of four months. Males accounted for 99.7% (897/900) of the study population. The mean age of donors was 29.7(SD +7.9) years with the age group 18-29 years being the most prevalent and ≥50 years being the least prevalent. Various baseline characteristics of blood donors are shown in Table-I. Regarding history of symptoms, 24.2% blood donors had experienced SARS-CoV-2 related symptoms while 75.7% did not report any symptom.

An overall seroprevalence of SARS-CoV-2 IgG antibody was detected as 20% (n=180/900). Of these, seropositive donors who did not report any SARS-CoV-2 related symptom showed higher anti-SARS-COV-2 IgG seropositivity as compared to donors with history of COVID-19 related symptoms (14% versus 6%) (Table-II).

Table I: Baseline Sociodemographic, Clinical and Behavioral Characteristics of Blood donors for SARS-CoV-2 IgG Testing.

Variables	Categories	Donors Number (%)
Gender	**Male**	**897(99.7)**
	Female	**3(0.3)**
Age groups (years)	18-29	478 (53.1)
	30-49	404 (44.9)
	≥50	18 (2.0)
Educational Level	No higher education	690 (76.8)
	Higher education	210 (23.2)
COVID-19 related Symptoms	Yes	223 (24.6)
	No	677 (75.4)
Smoking	Yes	113(12.3)
	No	787(87.7)

Table II: Anti-SARS-CoV-2 IgG seroprevalence among blood donors		
Donors	Number (%)	SARS-Cov-2 IgG Seroprevalence Number (%)
NO COVID related symptoms	676 (75.4)	140 (14)
COVID related symptoms	224 (24.6)	60 (6)
Total	900 (100)	180 (20)

The correlation of SARS-COVID-2 IgG seropositivity with sociodemographic and behavioral characteristics of blood donors is shown in Table-III. Younger blood donors aged 18-29 years and lack of education in blood donors was found to be associated with increased seropositivity rate respectively (10.5 and 14.5%). However, age and educational background was not significantly associated with SARS-CoV-2 IgG seropositivity (P=0.4 and P=0.2). Non-smokers showed 19.3% SARS-CoV-2 IgG seroprevalence rate as compared to smokers (P=0.2). Majority of the donors experienced COVID-19 related respiratory symptoms whereas the least common were gastrointestinal. In seropositive cases, the donors with respiratory symptoms were 6.2% and gastrointestinal 2.5% while among seronegative cases, 11.2% experienced respiratory and 4.2% gastrointestinal symptoms. Overall, fever, shortness of breath and anosmia/ageusia showed significant association with SARS-CoV-2 IgG seropositivity.

All the ELISA IgG negative samples tested for SARS-CoV-2 RNA were found out to be negative by PCR.

Table III: Seroprevalence of SARS-COV-2 IgG antibody with respect to various characteristics of blood donors			
Characteristics	**Tested N (%)**	**SARS-COV-2 IgG antibody N (%)**	
		Yes	**No**
Age Groups			
18-29	478 (53.1)	94 (10.5)	384 (42.6)
30-49	404 (44.9)	81 (9)	323 (35.9)
≥50	18 (2.0)	5 (0.5)	13 (1.5)
Edu. Level			
No education	690 (76.8)	131(14.5)	559 (62.1)
Educated	210 (23.2)	49 (5.4)	161 (17.8)
Smoking			
Yes	113 (12.3)	6 (0.6)	
No	787 (87.7)	107 (11.8)	
		174 (19.3)	
		613 (68.1)	

Discussion

Sero-epidemiological studies are needed because of the ongoing COVID-19 pandemic, which assists in evaluating the community spread of SARS-COV-2 infection. Natural infection may result in the development of herd immunity, which can only be established if a large proportion of the general population is immune to COVID-19 infection. This curtails the transmission of the virus from an infected person to non-infected or susceptible individuals. Serological surveys may assist in the estimation of extent to which herd immunity prevails.[1] The current study was conducted in the Northern region of Pakistan to illustrate the seroprevalence of SARS-CoV-2 IgG antibody by ELISA and associated risk factors among blood donors in the late phase of pandemic, because the later the period, the higher could be the prevalence.

Our study showed presence of IgG in 20% blood donors which is relatively lower than the 37.8% reported in a previous study from southern city of Pakistan. Despite exclusion of previously symptomatic cases, a high rate was observed because this was the most affected region of Pakistan and small numbers of individuals were enrolled in this study due to which variation in prevalence may be observed.[5] A study from Guangzhou, China reported very low 0.09% prevalence of IgG antibody in only 2 out of 2199 voluntary blood donors. Therefore, they characterized blood donors as low risk population as compared to high-risk group such as health care workers for COVID-19 infection and also considered sampling time an influencing factor for low prevalence of IgG.[12] Similarly, studies from Wuhan and Shenzhen, China reported 2.2% and 0.02% SARS-CoV-2 IgG antibody positivity, respectively.[13] These findings have shown the effect of strict social restrictions and better surveillance systems in China as compared to our country. Another survey-based study from Italy showed 0.9% SARS-CoV-2 IgG seropositivity which is far less than reported in our study. However, study participants were recruited from a region with a moderate incidence of infection which might be responsible for low rate whereas in the highly affected region of Italy, Lombardy documented 23% IgG positivity rate.[14,15] The prevalence rate may vary due to the variations in the epidemiology of study population, stage of epidemic and methodology.

The gold standard for COVID-19 diagnosis RT-PCR is limited and asymptomatic SARS-CoV-2 infection makes blood safety worthy of consideration.

Asymptomatic individuals have very low viral RNA, usually ranges from 2 to 4 log copies per milliliter; it could be detected in whole blood, serum or plasma. Therefore, the theoretical possibility of viral transmission by blood cannot be overlooked.[16] In view of this, ELISA IgG negative samples (n=597) were tested for SARS-CoV-2 RNA by RT-PCR. None of the sample was found to be positive which is consistent with the findings of a study conducted in Italy.[15] A multi-centered study from China also reported no evidence of SARS-CoV-2 RNA among voluntary blood donors which is in agreement with the current findings regarding only absence of SARS-CoV-2 RNA.[17] All ELISA IgG negative samples were tested by using sensitive real time PCR assay of Roche Cobas 6800 system with better limit of detection as compared to other NAT methods and the results suggest that this virus may not appear a direct threat to blood safety.

Regarding substantial risk factors, cigarette smoking increases the risk of acquiring viral infections in a multifactorial way including suppression of the pulmonary immune function and alteration of muco-ciliary defense mechanisms. In our study, IgG seropositivity was found to be higher in healthy non-smokers as compared to smokers. This may be consistent with the notion that smoking is a risk factor for progression of the COVID-19 disease in individuals with underlying comorbidities.[18] The youngest blood donors aged from 18-29 years had shown higher IgG seropositivity rate. This was expected as youngest blood donors constitute the core workforce, are willing to be engaged in strenuous physical activities and thus more likely to be exposed to infection despite adhering to social distancing restrictions. Better knowledge and awareness on the preventive measures associated with SARS-CoV-2 infection may curb exposure to infection. High seropositivity was observed among donors lacking higher education, and this was predictable.

Our study has several limitations. Firstly, enrolled blood donors were majority males so SARS-CoV-2 IgG prevalence is not widely applicable to general population. Secondly, NAT-testing for SARS-CoV-2 active infection was not performed on nasal swabs which might have high viral load as compared to serum samples tested so far. Thirdly, we could not identify the neutralization activity and half-life of detected antibodies thus effectiveness of herd immunity cannot be established.

Conclusion

In conclusion, 20% of the blood donor population was found to be positive for SARS-CoV-2 IgG antibody which is a reflection of widespread asymptomatic virus circulation in our population.

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Open Access Original Article

The Effect of Serum Lactate Dehydrogenase (LDH) Level on the Response to Erythropoiesis Stimulating Agents in Patients with Low and Intermediate Risk Myelodysplastic Syndrome

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Abstract

Objective: To evaluate the effect of increased serum lactate dehydrogenase (LDH) level, known as a poor prognostic factor, on the response to erythropoiesis-stimulating agent (ESA) treatment in patients with low and intermediate-1 risk MDS –considered to be a group with good prognosis.

Methodology: We retrospectively identified 47 patients who were treated with ESA (epoetin-α or darbepoetin-α) due to low or intermediate risk MDS according to international prognostic scoring system (IPSS) the patients were evaluated from three different medical centers between 2006 and 2018. Patients' demographic, clinical and laboratory characteristics (including erythropoietin and LDH levels) were recorded and analyzed with respect to IPSS risk groups and the presence/absence of response to ESA.

Results: The low-risk group consisted of 32 patients, and the intermediate-1 group consisted of 15 patients. Thirty-three patients responded to ESA, while 14 did not. Survival analyses demonstrated that patients with low or normal LDH at baseline had longer survival than those with high LDH, and risk of death was increased by 8.868-fold in patients with high LDH. There was no relationship between LDH level and response to ESA therapy, but female gender increased the likelihood of ESA response by 9.19-fold.

Conclusion: Our findings show that LDH level is one of the predictable factor of survival among patients with MDS; however, it appears that baseline LDH is not associated with ESA response. Besides baseline erythropoietin levels were lower among ESA responders, logistic regression revealed that the only parameter associated with positive response to ESA was female gender.

Keywords: Lactate dehydrogenase, Myelodysplastic syndrome, Erythropoietin, Darbepoetin, Erythropoiesis-stimulating agent.

Introduction

Myelodysplastic Syndrome (MDS) is an acquired clonal disease in which myeloid cell maturation is impaired. Its primary feature may be considered the risk of leukemic transformation, while peripheral cytopenia may also occur due to ineffective hematopoiesis.[1,2] The clinical presentation is heterogeneous, with various types and combinations of cytopenia observed in patients, including anemia, which is present in around two-thirds of patients.[2] Treatment aims to reduce symptoms, prevent disease progression and death risk, and increase the quality of life. The decision on treatment modality is made according to the risk groups.[3]

The International Prognostic Scoring System (IPSS) is still widely used in the risk classification of patients with MDS based on three factors, the percentage of myeloblasts in the bone marrow, cytogenetic features, and the number of cytopenia types identified in peripheral blood.[4-6] According to the IPSS, MDS is divided into four prognostic categories according to risk: low, intermediate-1, intermediate-2, and high risk.[5] A revised form of the IPSS which has advanced classification does exist (IPSS-R)[7], but as mentioned before, the IPSS continues to be utilized in practice. Other prognostic factors have also been defined so far, including a high serum lactate dehydrogenase (LDH) level, which is now well accepted as a critical poor

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prognostic factor.[1] Additionally, the intermediate-1 risk group could be variable, and it has been shown that these patients demonstrate worse prognosis in the presence of elevated LDH. [8]

Erythropoiesis-stimulating agents (ESA), epoetin-α and darbepoetin-α, are frequently used to treat symptomatic anemia in patients with low and intermediate-1 risk, particularly in those without 5q deletion and patients who has low erythropoietin level (<500 IU/L).[9] Both of these agents are reported to have similar efficacy, but the relationship between response to ESA and LDH level is still unknown yet.

In this study, we aimed to evaluate whether increased serum LDH level, known as a poor prognostic factor, had any influence on the response to ESA treatment in patients with low and intermediate-1 risk MDS, who represent a group that is generally considered to have a good prognosis.

Methodology

We identified 47 patients who had been treated with ESA therapy from 2006 to 2018 in three tertiary referral hospitals in Turkey; Okmeydanı Training and Research Hospital, Ege University Faculty of Medicine Hospital, and İstanbul Kartal Prof. Dr. Lütfi Kırdar Training and Research Hospital. All subjects were retrospectively included through the evaluation of patient databases.

Diagnosis and evaluations: The MDS diagnosis of each patient was reevaluated and confirmed with respect to the MDS diagnostic criteria revised by Valent et al. in 2017.[10] These updated criteria consist of three major and three co-criteria, which are assessed when the patient has cytopenia of erythroid, myeloid or platelet series for at least 4 months –given that all other hematologic or non-hematologic causes of cytopenia can be excluded (pre-requisite). In addition of pre requisite the presence of one major criterion is accepted to yield a diagnosis of MDS. In the event that no major criteria are met, the presence of at least two co-criteria can confirm MDS diagnosis.[10]

Major criteria: (i) presence of at least 10% dysplasia in at least one of the erythroid, myeloid or megakaryocytic series, (ii) >15% ring sideroblasts in bone marrow smear or >5% in the presence of SF3B1 mutation, (iii) myeloblast proportion of 5–19% in bone marrow smear or 2–19% in peripheral blood smear. Co-criteria: (i) histology or immunohistochemistry abnormalities in bone marrow biopsy, (ii) myeloid and/or erythroid monoclonal population presence indicating multiple MDS-related phenotypic aberration in the immunophenotypic flow cytometry analysis of bone marrow cells, (iii) identification of MDS-related mutations with molecular sequencing studies of clonal populations of myeloid cells.

Among the patients who met the diagnostic criteria, only those who were categorized as low risk or intermediate-1 risk according to the IPSS were included in the study.[6] The type of MDS was also determined and recorded according to the French-American-British (FAB) classification due to historical importance: RA (Refractory anemia), RARS (refractory anemia with ring sideroblasts), RCMD (refractory cytopenia with multilineage dysplasia), and RCMD-RS (Refractory cytopenia with multilineage dysplasia, ring sideroblasts ≥15%).[11]

Demographic and clinical characteristics, including age, gender, comorbidities (diabetes mellitus, hypertension, coronary artery disease, cerebrovascular accident, chronic obstructive pulmonary disease, kidney diseases, thyroid diseases and hematologic diseases), duration with MDS diagnosis and treatments received, and red blood cell (RBC) transfusion requirements were recorded. The laboratory characteristics of patients consisted of total blood count, beta-2 microglobulin, erythropoietin (EPO) and lactate dehydrogenase (LDH) levels. In addition to recording quantitative values for LDH, we also categorized patients according to LDH level into three groups: low, normal and high.

Administration of ESA: All individuals included in the current study had received either epoetin-α or darbepoetin-α treatment. Epoetin was administered at a weekly dose of 30.000 IU, and darbepoetin was administered at a dose of 150 µg applied once every weeks.

Assessment of treatment response: Patients' response to ESA treatment was determined via the revised 2018 criteria of the International Working Group.[12] According to their transfusion dependency, patients were divided into three categories to assess response with respect to their defined status (transfusion-independent, low transfusion-dependent and high transfusion-dependent). The definitions for each of the transfusion-dependency groups and their response to treatment were as follows:

After treatment with ESA agents, both risk groups showed significant increase in hemoglobin level (p=<0.001 for low risk group and p=0.013 for intermediate-1 risk group, data was shown in table I). The LDH level in low-risk group increased after treatment, while the intermediate-1 risk group had similar LDH levels at pre- and post-treatment.

i) Patients who did not need a transfusion during the 1–16-week observation period were considered transfusion independent, and an increase of at least 1.5 g/dl in hemoglobin during the 16-week follow-up was accepted as positive response to treatment.

ii) During the 16-week follow-up, the patients who needed a total of 3-7 units of RBC transfusion on at least two occasions were considered low transfusion-dependent patients. Among these, during the 16–24-week follow-up period, the absence of transfusion was accepted as a positive response.

iii) Patients who needed a total of 8 units or more RBC transfusion on at least two occasions during the 16-week follow-up were considered to be high transfusion-dependent. Individuals who did not require transfusion during the 16–24-week follow-up were accepted to have major response, whereas minor response was defined among patients with a 50% reduction in transfusion need.

Apart from these three groups, patients who needed 1-2 units of RBC transfusion in 16 weeks were not included in any transfusion-dependency category, and positive response was identified as a hemoglobin increase of at least 1.5 g/dl in this group of individuals.

Statistical analyses were conducted with the SPSS software (version 21.0, IBM, Armonk, NY, USA). Comparison of categorical variables was performed with Pearson Chi-square tests. The normality of distribution of continuous variables was tested with the Shapiro-Wilk test and Q-Q histograms, and comparisons were performed with the independent samples t-test or the Mann-Whitney U test, in parametric and non-parametric continuous variables, respectively. Depiction of continuous variables was performed using the median and inter-quartile range (IQR), while categorical data were depicted with count (n) and percentage (%).

Multivariable analyses were conducted by the inclusion of parameters that demonstrated significant differences in univariate analyses to determine parameters independently associated with various characteristics (such as treatment response and survival). The Log-rank method was applied in the analysis of survival. P-values of 0.05 or lower were considered to be statistically significant.

Results

The median age was 69.5 (range, 34–85) years, and 14.8% of patients were male. The characteristics of patients which depend on IPSS risk groups (low, intermediate-1) are described in Table I. The low-risk group consisted of 32 patients, and the intermediate-1 group comprised 15 patients. The median EPO level was 31.8 (4.40 – 303.5)mIU/mL in overall and it was similar in low and intermediate-1 risk groups (28.0 vs 37.8, respectively p=0.344), 17% of the patients were transfusion-independent. Comparisons based on risk groups showed that the low-risk group had a significantly higher frequency of female gender (p=0.026). These two groups were also significantly different in terms of white blood cell count (WBC) (5.88 (3.69 – 10.62) vs 3.38 (2.14 – 8.44), p=0.003) and neutrophil levels (3.79 (0.69 – 8.02) vs. 1.83 (0.97 – 4.61), p=0.015) at the time of diagnosis. All patients had low hb level (<10 g/dl) when ESA therapy was started. Gender distribution according to ESA response is shown in Figure 1.

Therapy response: Epoetin and darbepoetin were used in 38 (81%) and 9 (19%) patients, respectively. Response rates were similar in the epoetin (n = 27; 71%) and darbepoetin (n = 6; 67%) treated groups (p = 1). Overall, 33 patients showed a response to treatment, and the median response duration was 37 (IQR: 7–119) months. When treatment groups were compared, almost all characteristics were similar among the groups, the only differences was: and follow-up duration was longer in the epoetin group compared to the darbepoetin group (48 [9–158] months vs. 16 [7–62] months, p = 0.005). After treatment, patients in both the epoetin and darbepoetin groups demonstrated a statistically significant increase in hemoglobin level (p <0.001 and p = 0.021, respectively). Additionally, after treatment, the epoetin group showed a significant increase in LDH (p = 0.026), whereas the change was non-significant in the darbepoetin group (p = 0.214).

When individuals with (n = 33) and without (n = 14) response to ESA treatment were compared, we found that female frequency was higher and baseline

Table I: Summary of patient characteristics and analysis results according to IPSS groups				
	IPSS groups			
	Low (n=32)	Intermediate-1 (n=15)	Overall (n=47)	P value
Age	70 (34 – 85)	69 (52 – 81)	69.5 (34 – 85)	1.000
< 60	5 (16.13%)	3 (20.00%)	8 (17.39%)	1.000
≥ 60	26 (83.87%)	12 (80.00%)	38 (82.61%)	
Gender				
Male	2 (6.25%)	5 (33.33%)	7 (14.89%)	0.026
Female	30 (93.75%)	10 (66.67%)	40 (85.11%)	
Type of MDS				
RA	32 (100.00%)	12 (80.00%)	44 (93.62%)	0.077
RARS	0 (0.00%)	1 (6.67%)	1 (2.13%)	
RCMD	0 (0.00%)	1 (6.67%)	1 (2.13%)	
MDRS	0 (0.00%)	1 (6.67%)	1 (2.13%)	
Comorbidities	22 (70.97%)	12 (80.00%)	34 (73.91%)	0.723
Accompanying malignancy	3 (9.38%)	1 (6.67%)	4 (8.51%)	1.000
Splenomegaly	1 (3.23%)	2 (13.33%)	3 (6.52%)	0.244
Lymphadenopathy	0 (0.00%)	0 (0.00%)	0 (0.00%)	N/A
Abnormal cytogenetics	1 (3.13%)	4 (26.67%)	5 (10.64%)	0.030
Genetic mutation	3 (9.38%)	2 (14.29%)	5 (10.87%)	0.633
Erythropoietin	28.0 (4.4 – 303.5)	37.8 (5.1 – 297.0)	31.8 (4.40 – 303.5)	0.344
Hemoglobin at diagnosis	9.75 (6.10 – 11.72)	9.70 (5.60 – 11.00)	9.70 (5.60 – 11.72)	0.991
WBC at diagnosis (x1000)	5.88 (3.69 – 10.62)	3.38 (2.14 – 8.44)	5.46 (2.14 – 10.62)	0.003
Neutrophil at diagnosis (x1000)	3.79 (0.69 – 8.02)	1.83 (0.97 – 4.61)	3.52 (0.69 – 8.02)	0.015
Platelet at diagnosis (x1000)	226 (17.9 – 434)	199 (70 – 401)	208 (17.9 – 434)	0.708
Beta-2 microglobulin	365.5 (193 – 1650)	632 (171 – 3443)	426 (171 – 3443)	0.169
Anemia	31 (96.88%)	15 (100.00%)	46 (97.87%)	1.000
Leukopenia	3 (9.68%)	8 (53.33%)	11 (23.91%)	0.002
Leukocytosis	1 (3.23%)	0 (0.00%)	1 (2.17%)	1.000
Thrombocytopenia	4 (12.90%)	5 (33.33%)	9 (19.57%)	0.127
Pancytopenia at diagnosis	2 (6.25%)	2 (13.33%)	4 (8.51%)	0.583
Bicytopenia at diagnosis	5 (16.67%)	7 (46.67%)	12 (26.67%)	0.070
Transfusion dependency				
Low	1 (3.13%)	1 (6.67%)	2 (4.26%)	0.848
High	4 (12.50%)	2 (13.33%)	6 (12.77%)	
Erythropoiesis-stimulating agent				
Epoetin	25 (78.13%)	13 (86.67%)	38 (80.85%)	0.697
Darbepoetin	7 (21.88%)	2 (13.33%)	9 (19.15%)	
Response to ESA				
Absent	9 (28.13%)	5 (33.33%)	14 (29.79%)	0.742
Present	23 (71.88%)	10 (66.67%)	33 (70.21%)	
Hemoglobin				
Before treatment	9.54 (6.10 – 10.80)	9.60 (7.30 – 10.80)	9.54 (6.10 – 10.80)	0.991
After treatment	11.70 (7.79 – 14.70)	10.80 (7.80 – 12.90)	11.70 (7.79 – 14.70)	0.213
p (within variables)	<0.001	0.013	<0.001	
Change in hemoglobin	2.02 (-0.07 – 4.60)	1.40 (-2.90 – 3.80)	1.80 (-2.90 – 4.60)	0.126
LDH				
Before treatment	191 (121 – 431)	182 (119 – 305)	190 (119 – 431)	0.632
After treatment	212 (122 – 416)	174 (128 – 279)	200 (122 – 416)	0.349
p (within variables)	0.016	0.443	0.016	
LDH categories				
Before treatment				
Low	3 (9.38%)	2 (13.33%)	5 (10.64%)	0.848
Normal	23 (71.88%)	11 (73.33%)	34 (72.34%)	
High	6 (18.75%)	2 (13.33%)	8 (17.02%)	
After treatment				
Low	3 (9.38%)	0 (0.00%)	3 (6.38%)	0.074
Normal	17 (53.13%)	13 (86.67%)	30 (63.83%)	
High	12 (37.50%)	2 (13.33%)	14 (29.79%)	
p (within variables)	0.058	0.317	0.033	
Transformation to acute leukemia	0 (0.00%)	0 (0.00%)	0 (0.00%)	N/A
Follow-up time (months)	44.5 (7 - 158)	44 (9 - 108)	44 (7 - 158)	1.000
Status				
Alive	28 (87.50%)	13 (86.67%)	41 (87.23%)	1.000
Exitus	4 (12.50%)	2 (13.33%)	6 (12.77%)	
Data are given as median (minimum-maximum) for continuous variables according to the normality of distribution and as frequency (percentage) for categorical variables				

Table II. Summary of patient characteristics and analysis results with regard to ESA response

	Response to ESA			
	Absent (n=14)	Present (n=33)	Total	**p**
Age	69.5 (34 - 80)	69.5 (44 - 85)	69.5 (34 – 85)	0.839
< 60	2 (14.29%)	6 (18.75%)	8 (17.39%)	1.000
≥ 60	12 (85.71%)	26 (81.25%)	38 (82.61%)	
Gender				
Male	5 (35.71%)	2 (6.06%)	7 (14.89%)	0.018
Female	9 (64.29%)	31 (93.94%)	40 (85.11%)	
Type of MDS				
RA	12 (85.71%)	32 (96.97%)	44 (93.62%)	0.153
RARS	1 (7.14%)	0 (0.00%)	1 (2.13%)	
RCMD	1 (7.14%)	0 (0.00%)	1 (2.13%)	
MDRS	0 (0.00%)	1 (3.03%)	1 (2.13%)	
IPSS group				
Low	9 (64.29%)	23 (69.70%)	32 (68.09%)	0.742
Intermediate-1	5 (35.71%)	10 (30.30%)	15 (31.91%)	
Abnormal cytogenetics	1 (7.14%)	4 (12.12%)	5 (10.64%)	1.000
Genetic mutation	1 (7.14%)	4 (12.50%)	5 (10.87%)	1.000
Erythropoietin	60.1 (6.1 - 297)	23 (4.4 - 303.5)	31.8 (4.40 – 303.5)	0.023
Hemoglobin at diagnosis	9.15 (5.60 - 11.10)	9.80 (6.10 - 11.72)	9.70 (5.60 – 11.72)	0.122
WBC at diagnosis (x1000)	5.37 (2.14 - 9.03)	5.46 (2.84 - 10.62)	5.46 (2.14 – 10.62)	0.346
Neutrophil at diagnosis (x1000)	2.71 (1.29 - 4.50)	3.74 (0.69 - 8.02)	3.52 (0.69 – 8.02)	0.223
Platelet at diagnosis (x1000)	196 (17.9 - 362)	216.5 (40 - 434)	208 (17.9 – 434)	0.599
Erythropoiesis-stimulating agent				
Epoetin	11 (78.57%)	27 (81.82%)	38 (80.85%)	1.000
Darbepoetin	3 (21.43%)	6 (18.18%)	9 (19.15%)	
G-CSF usage	0 (0.00%)	1 (3.03%)	1 (2.13%)	1.000
Hemoglobin				
Before treatment	9.35 (7.30 – 10.80)	9.54 (6.10 – 10.80)	9.54 (6.10 – 10.80)	0.780
After treatment	10.13 (7.80 – 12.20)	11.80 (7.79 – 14.70)	11.70 (7.79 – 14.70)	0.002
p (within variables)	0.048	<0.001	<0.001	
Change in hemoglobin	0.99 (-2.90 – 3.32)	2.20 (0.17 – 4.60)	1.80 (-2.90 – 4.60)	<0.001
LDH				
Before treatment	210 (135 – 305)	181 (119 – 431)	190 (119 – 431)	0.170
After treatment	223.5 (122 – 416)	191 (128 – 318)	200 (122 – 416)	0.340
p (within variables)	0.433	0.019	0.016	
LDH categories Before treatment				
Low	0 (0.00%)	5 (15.15%)	5 (10.64%)	0.293
Normal	11 (78.57%)	23 (69.70%)	34 (72.34%)	
High	3 (21.43%)	5 (15.15%)	8 (17.02%)	
After treatment				
Low	1 (7.14%)	2 (6.06%)	3 (6.38%)	0.986
Normal	9 (64.29%)	21 (63.64%)	30 (63.83%)	
High	4 (28.57%)	10 (30.30%)	14 (29.79%)	
p (within variables)	1.000	0.011	0.033	
Transformation to acute leukemia	0 (0.00%)	0 (0.00%)	0 (0.00%)	N/A
Follow-up time (months)	55.5 (8 - 158)	37 (7 - 119)	44 (7 - 158)	0.205
Status				
Alive	11 (78.57%)	30 (90.91%)	41 (87.23%)	0.344
Exitus	3 (21.43%)	3 (9.09%)	6 (12.77%)	
BM blasts at diagnosis, %; median	0 (0-3)	0 (0-2)	0 (0-2)	0.190
RBC transfusion at baseline	3 (21.4%)	5 (15.2%)	8 (17%)	0.601
BM fibrosis, n (%)	7 (50.0%)	26 (78.8%)	33 (70.2%)	0.048

erythropoietin levels were lower in responders; all other characteristics were similar at baseline. After treatment, a significant increase in hemoglobin level was observed in both non-responders (p = 0.048) and responders (p <0.001). However, the amount of Hb increase was significantly greater among responders (p <0.001) and these patients also had significantly higher Hb level after treatment when compared to non-responders (p = 0.002). Pre- and post-treatment LDH levels were similar in non-responders, but a significant increase was observed in responders (p = 0.019) (Table II). Response to ESA according to risk groups and ESA agents (darbepoetin vs. epoetin) are shown in Figure 2 and Figure 3, respectively. Patients' distribution to LDH categories according to ESA response is shown in Figure 4.

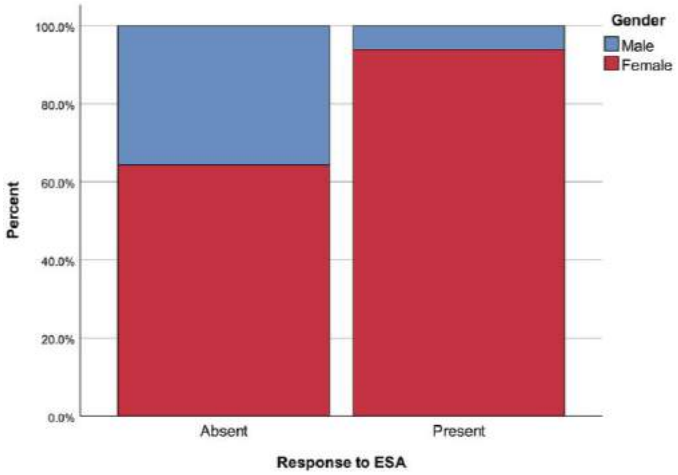

Figure 1. Gender distribution with respect to ESA response groups

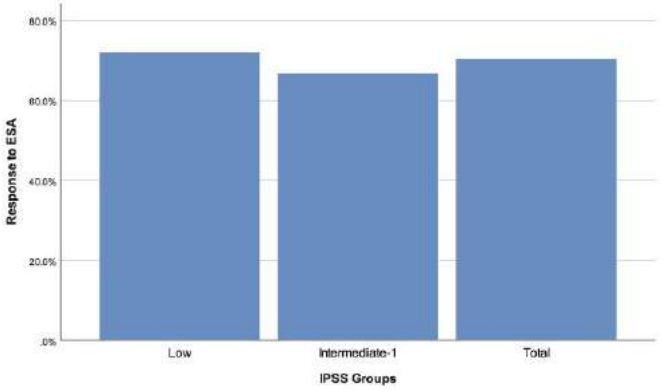

Figure 2. Response to ESA according to IPSS Groups

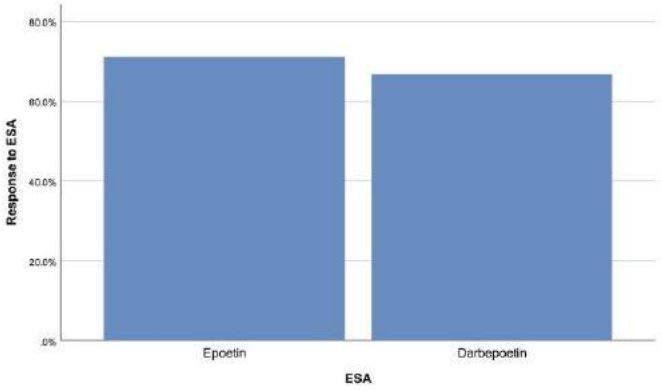

Figure 3. Response to ESA according to treatment type

Survival functions based on LDH categories (low or normal versus high) demonstrates that patients with low or normal LDH had longer survival (Figure 5). The cumulative survival analysis is shown in Figure 6. We performed Cox regression analysis to determine

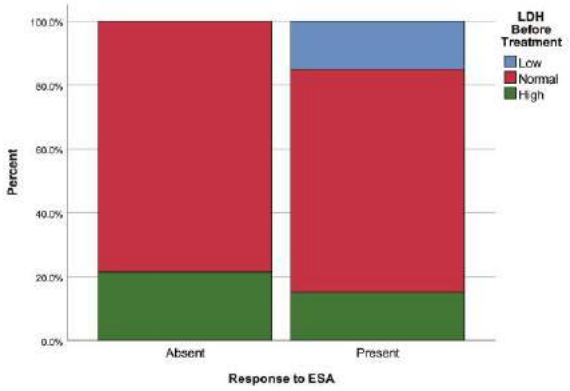

Figure 4. Comparison of patients' distribution to LDH categories with respect to ESA response

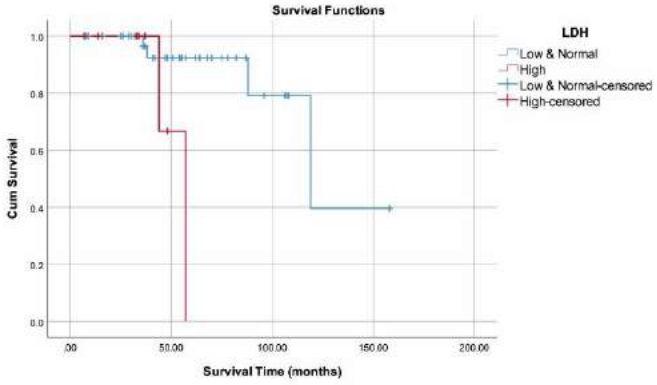

Figure 5. Survival functions with respect to LDH categorization (low or normal LDH vs. high LDH)

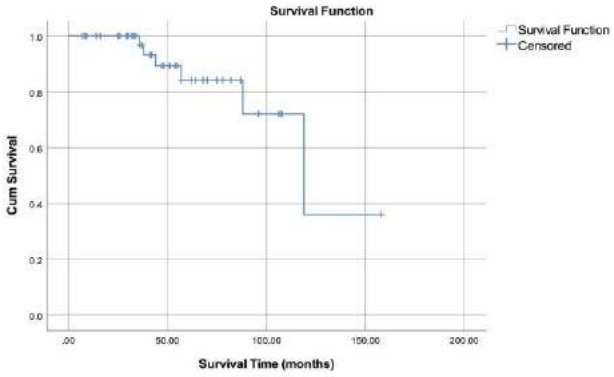

Figure 6. The Cumulative Survival Function

significant prognostic factors in MDS. Patients with high LDH values had 8.868-fold higher risk of death compared to other patients (HR: 8.868, 95% CI: 1.214–64.751, p = 0.031). Other variables included in the model, age (p = 0.066), gender (p = 0.150), IPSS group (p = 0.570), transfusion dependency (p = 0.079), ESA (p = 0.067) and response to ESA (p = 0.509) were found to be non-significant. We also performed logistic regression to determine factors that were influential on treatment

response. We included gender and baseline EPO values in the model because they were the only variables with a p-value below 0.100 in univariate analysis. Females were found to have a 9.19-fold greater likelihood of demonstrating positive response to ESA treatment (OR: 9.19, 95% CI: 1.47–57.39, p = 0.018), whereas baseline EPO levels were non-significant (p = 0.093).

Discussion

This study demonstrated that patients with low-risk and intermediate-1-risk MDS (according to IPSS) had similar characteristics at baseline, except for gender distribution and WBC and neutrophil counts. The comparison of patients classified as 'responders' or 'non-responders' (regarding ESA treatment) showed that both groups had a significant increase in Hb level after ESA administration, while the LDH increase was only significant among responders. Cox regression analysis revealed that patients with high LDH at baseline had an 8.868-fold higher risk of death compared to those with low or normal LDH. This finding is in agreement with prior studies which have shown that LDH level is a prognostic factor in MDS. We did not find any relationship between baseline LDH levels and response to ESA treatment. The only parameter that affected treatment response was gender, with females having a 9.19-fold greater likelihood of responding to ESA therapy.

The majority of patients at low or intermediate-1 risk had an erythroid response after treatment with ESA, consistent with previous studies.[13-15] Transfusion dependence and iron overload have been linked with poor survival and worse health outcome in MDS, including cardiovascular, hepatic, and endocrine dysfunctions.[16] Achieving erythroid response is critical; because it has been shown that reducing transfusion-related incidents in MDS patients can have a significant survival impact that differs by risk stratification, with median survival extending to three years in low-risk patients while it can remain as low as two months in high-risk patients.[17] Our results agreed with contemporary literature, as demonstrated by the significant increases in Hb level in patients with positive response to ESA (either epoetin or darbepoetin). Similarly, a previous meta-analysis reported that epoetin and darbepoetin yielded similar erythroid response rates in anemic MDS patients.[18]

There was no relationship between LDH level and response to ESA therapy. Although, improvement of Hh level was significantly higher among responders ($p<0.001$) Hb level was increased among both the responders and non-responders after treatment. Noteworthy, this finding is confounded by the fact that the assessment of treatment response includes threshold values for hemoglobin change (1.5 g/dl). The frequency of females was higher and baseline EPO levels were lower among responders, suggesting a relationship between ESA response and these two parameters. Logistic regression revealed that female gender was associated with positive response to ESA, while EPO levels did not influence the likelihood of ESA response.

Various other studies have also attempted to correlate clinical outcomes with LDH levels in MDS. In a study evaluating hypomethylating agent (HMA) therapy, Coston et al. found that HMA response was correlated with lower serum LDH levels.[19] In another study, Moon et al. revealed that high LDH levels were significantly associated with worse survival in patients receiving azacitidine.[20] Similarly, Park et al. also obtained a result indicating that increased levels of LDH had negative effects on patient survival in addition to age.[21] Although we also found a relationship between high LDH level and survival, our results showed a lack of relationship between ESA response and LDH level.

The current study demonstrated that patients with low or normal LDH levels had a significantly extended duration of survival. Interestingly, our patients' overall survival duration was 117.66 (92.06–143.26) months, a value that exceeds the results reported by the majority of the literature on this topic (ranging from 3.1 to 37 months).[20, 22-24] This controversial result may be associated with various factors, including patient-based and treatment-dependent differences; however, the extreme difference in survival warrants further studies in which the possible phenotypical differences can be assessed among patients with MDS. Considering the previously reported role of LDH levels in patients with MDS and the fact that we found it to be the most important factor associated with survival, we believe that future studies would benefit from prospective analyses that employ patient stratification based on not only treatment characteristics, but also other parameters, including LDH levels, race, genetic factors and related efficacy of different treatment modalities.

Recent changes in classification have resulted in the use of the 5-group IPSS-R (revised) for risk assessment in MDS, which enables a reliable categorization in terms of length of survival and risk of leukemic transformation. However, the approach to treatment does not differ with each of the 5 risk groups, and it has been noted that research is required for the elucidation of this problem. [7] The last issue that has to be noted is that there is no pharmaceutical agent licensed according to IPSS-R classification. So, when deciding to use a certain treatment, current approaches require the consideration of FAB criteria and IPSS criteria. For these reasons, we did not re-classify patients with respect to IPSS-R in this study. Newman et al. reported that growth factors such as G-CSF and EPO provided substantial advantages in treating patients with MDS, but there were concerns regarding the application of thrombopoietic growth factors in patients with MDS.[25]

Finally, one of the most notable findings of this study was the fact that both responders and non-responders had significant improvement in Hb after treatment. Even though the increase in Hb and the post-treatment comparison between groups indicate a greater change in the responder group compared to non-responders, this finding may raise questions pertaining to the definition of response to ESA treatment in patients with MDS. Previous studies have also indicated that there may be a need for new approaches to determine ESA response and have proposed different methods throughout the last twenty years.[26-29] In the face of such limitations, we suggest that future studies should assess ESA response based on more than one method or scoring system.

Conclusion

In summary, our results support current evidence that high LDH levels at baseline are associated with poor survival in low- and intermediate-1-risk patients with MDS. Although the amount of increase in Hb was superior among responders compared to non-responders and the fact that post-treatment comparisons showed higher values among responders, this is highly likely to be a direct result of the response classification utilized in this study (which includes hemoglobin increase). Additionally, contrary to our hypothesis, LDH levels were not found to be associated with the response to ESA therapy; in fact, responders had a significant increase in LDH after treatment, whereas non-responders did not demonstrate any change. Additionally, female gender appears to be associated with a greater likelihood of benefitting from ESA therapy. These conclusions require extensive studies for confirmation, especially those including patient stratification based on LDH levels, gender and other parameters that could cause variations in treatment response.

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Open Access Original Article

Molecular Screening for Malaria Parasitemia among Blood Donors: A Regional Transfusion Center Study in Pakistan

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Abstract

Objective: This study was carried out to determine asymptomatic malaria parasitemia among blood donors using molecular method and frame guidelines for malaria screening in blood banks of Northern Pakistan.

Methodology: In this cross-sectional study, a total of 1000 asymptomatic blood donors were enrolled between April and September, 2019. DNA extraction, amplification and detection of targeted gene (18S small subunit rRNA) was carried out using DNA extraction kit and Real Time PCR system respectively. Any sample found positive was retested for confirmation. Malaria parasitemia was expressed as percentage using descriptive statistics.

Results: Among 1000 asymptomatic donors, all donors were males with a median age of 30.0 years. Malarial parasite was detected in 0.1% blood donors (n=1/1000). Upon retesting of positive sample by another RT-PCR system, consistent results were obtained. Donor questionnaire did not reveal any history of previous exposure.

Conclusion: Malaria appears to be very uncommon in asymptomatic male blood donors and screening by NAT would not be economical. Adding effective donor screening criteria is suggested.

Keywords: Blood safety, Malaria, Molecular testing.

Introduction

Malaria inflicts global population and an estimated 228 million new cases are reported worldwide.[1] Approximately, 60% of Pakistan population contributes to malaria burden with annual parasitic incidence (API) rate of 1.8.[2] Apicomplexan parasites of the genus *Plasmodium* are the etiological agent which disseminates by bite of infected female Anopheles mosquito during a blood meal. Most common species involved are the *Plasmodium vivax* and *Plasmodium falciparum*. Besides, transfusion of blood components contaminated with malarial parasites is also a contributing source.[3,4]

Transfusion transmitted malaria (TTM), first described by Woosley in 1911[5], has gained interest in blood banking because of conversion of hemoglobin into dysfunctional methemoglobin leading to decreased systemic delivery of oxygen accompanied by fatal complications in debilitated recipients.[6] Contaminated blood products, especially whole blood and red cell concentrates, directly release the merozoites, in the bloodstream leading to adverse outcomes as compared to vector-borne transmission where pre-erythrocytic phase confers advantage in triggering the development of protective immunity.[7]

Survival of parasites for at least 18 days at storage temperature of blood products is a well-recognized risk of TTM.[8] Microscopy is a gold standard method however, sub-patent infections characterized by low parasite density (<10 parasites/μL) escapes detection which remains a diagnostic challenge in blood banks.[9,10] Thus, appropriate screening tools are the mainstay of prevention of TTM. High-sensitive molecular methods need to be employed to detect low infectious inoculum in asymptomatic blood donors which act as reservoirs sustaining malaria transmission.[11,12] As far as we know, there is lack of data depicting the actual burden of malaria prevalence among blood donors in Pakistan. Therefore, this study was conducted using highly sensitive method such as polymerase chain reaction (PCR) to identify the risk of and burden of TTM.

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Methodology

Transfusion center based cross-sectional study was conducted from April to September 2019. Ethics Committee of the Armed Forces Institute of Transfusion (AFIT) approved the research protocol. Prospective random donors with age ranges from 18-65 years and body weight ≥ 50kg were recruited using structured questionnaire while those who were seropositive for any transfusion transmissible infections (TTIs) including, HIV, HBV, HCV and syphilis, having history of anemia, bleeding disorders, body weight <50kg and refused to participate were excluded. Written informed consent was obtained from all enrolled donors. A total of 1000 asymptomatic blood donors were included by using random sampling technique. A 5ml of whole blood sample was collected into ethylene diamine tetra acetate (EDTA) tubes from each donor following standard protocol for venipuncture. DNA was extracted using commercial DNA-extraction kit (Qiagen, Germany) according to manufacturer's instructions for real time-PCR diagnosis of malaria. DNA amplification and detection were carried out using Applied Biosystems ABI 7500 Real-Time PCR System (Thermo Fisher Scientific, USA). Primers and probe based on detecting conserved sequences of 18S small-subunit ribosomal ribonucleic acid (rRNA) genes of all *Plasmodium* species are shown in Table I. Negative and positive controls were run with each batch. Any sample found positive was retested in duplicate by Rotor gene plex RT-PCR system for confirmation and re-examination of the questionnaire filled by malaria infected donors was also performed.

Table I: Primers and Probes for detection of Malarial Parasites

Primers	Sequence
Forward	5′-ACATGGCTATGACGGGTAACG-3′
Reverse	5′-TGCCTTCCTTAGATGTGGTAGCTA-3′
Probe TaqMan®	5′-TCAGGCTCCCTCTCCGGAATCGA-3′
Reporter Dye	FAM (carboxyfluorescein) on 5′ end
Quencher dye	BHQ1 (black whole quencher) on 3′ end

Descriptive analysis was carried out using Statistical Package for Social Sciences (SPSS) version 23.0. Parasitemia was expressed as percentage (positive samples/Total samples ×100).

Results

A total of 1000 blood donors were screened for malarial parasites using RT-PCR method. Demographic characteristics showed that all donors were males and their median age was 30.0 years (ranging from 18-65 years). Highest proportion was constituted by age group 18-28 years (Table II). Among these, only one asymptomatic donor (0.1%, n=1/1000) was found to be positive for malarial parasite (Figure 1). This case was also tested in duplicate for confirmation using another RT-PCR system. Results obtained by two RT-PCR methods were consistent. Upon re-examination of the questionnaire of the malaria positive donor, no history of previous possible exposure was identified.

Table II: Demographic characteristics of enrolled donors

Variables	N(%)
Gender	
Male	1000(100%)
Female	00(0%)
Age Groups	
18-28 years	550 (55%)
29-39 years	350 (35%)
≥40 years	100 (10%)

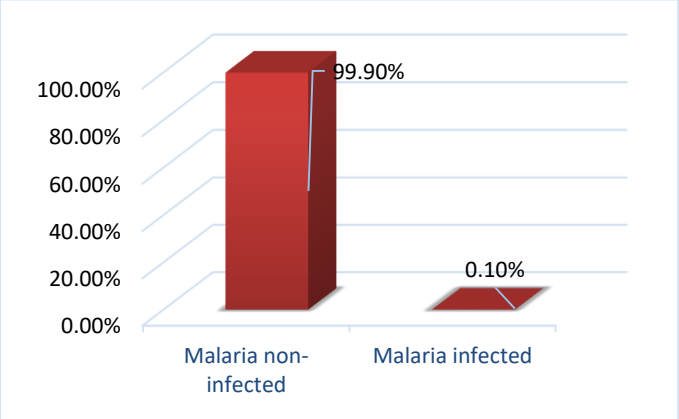

Figure 1. Distribution Frequency of Malaria Infected and Non-infected Blood donors.

Discussion

Blood transfusion remains a major risk factor in transmission of various infections. Blood transfusion services employ various serological and molecular methods to detect the presence of TTIs in order to provide safe and effective blood.[13] Pakistan is epidemiologically a moderate-malaria endemic country

Table III: Comparison of current study with previously conducted local studies

Year of Study	Place of Study	Method	TTM Prevalence	Reference No.
2019-2020	**Rawalpindi**	**RT-PCR**	**0.1%**	**present**
2018-2019	Faisalabad	CLIA	0.89%	16
2016-2018	Multan	ICT	0.0%	15
2015-2016	Lahore	ICT	0.39 %	17

ICT; Immunochromatographic technique, CLIA; Chemiluminescence microparticle immunoassay, RT-PCR; Real time polymerase chain reaction

with varying degree of endemicity in its different provinces with least epidemiology of 1.1% reported in Northern Pakistan in 2017.[3] Malaria screening is mostly performed by light microscopy and rapid detection test (RDT) which are unable to detect low parasitemia thus accurate figures of TTM prevalence needs to be investigated using highly sensitive methods in transfusion medicine. Accurate diagnosis and management of malaria is essential to avoid post-transfusion hemolysis.[14] In the present study, 0.1% asymptomatic donor population was found positive using RT-PCR method. Prevalence varies across different regions of Punjab Province, Pakistan as shown in Table III.[15-17]

To date, this was the first study which employed PCR-based testing of *Plasmodium* pathogens among blood donors. High sensitivity of PCR should be taken into consideration because sub-patent blood donors serve as a reservoir of transmission. Light microscopy of stained blood smears is widely used method in clinical diagnostic laboratories but time-consuming (15-20 minutes per microscopic observation) and laborious procedure restricts its utilization to screen large population in blood banks. Moreover, its detection limit is 50 parasites/ul whereas most asymptomatic donors have lesser parasitemia.[18] In contrast to international studies, a retrospective study conducted in India showed 0.024% prevalence rate for malarial antigen by RDT method.[19]

African countries like Ghana and Nigeria reported approximately 8% prevalence of malarial parasites due to their favorable climate and increased number of vector-mosquitoes.[20,21] Another study from Saudi Arabia observed 0.0% positivity rate for malarial antigens in endemic and non-endemic regions.[22,23] However, actual prevalence could be high because both antigen detection tests or microscopy cannot detect low parasite density. A study from Eastern China reported presence of malarial antibodies in 2.13% of blood donors.[24] In Malaysia, 33.6% prevalence of malaria was detected which is in disagreement with the current study.[25] Molecular screening was carried out on 400 healthy blood donors in Egypt to detect ssrRNA gene of *Plasmodium* but no subclinical malarial infection was detected using PCR which is comparable with the current study. This was attributed to implementation of effective donor selection criteria as recommended by World Health Organization (WHO).[26]

Donor screening serves as a fundamental step in enhancing safety and quality of blood and blood products thereby ensuring the deferral of unhealthy donors.[14] The current study highlights the proper usage of structured questionnaire along with verbal screening based on identification of malaria risk factors in regions with low endemicity. Moreover, staff should be trained and vigilant enough to recognize and explain the questions to donor if they found any difficulty in understanding due to low literacy levels, which is a well-recognized issue in developing countries. This is also economical because donors are deferred at this stage which subsequently reduces the wastage of resources such as consumables, screening tests and saves donor and staff time. Malaria infected donor was lost to follow up so in our opinion, it might be possible that donor did not comprehend the questions or deliberately provided inaccurate information owing to fear of deferral. Hence, absence of information regarding previous exposure or malaria risk factors was noted upon retrieval of donor questionnaire. The present investigation also revealed that only male blood donors attended the transfusion center. This could be attributed to social factors associated with female donation followed by deferral due to anemia, lactation or pregnancy which encapsulated the exclusion criteria. The PCR-based detection of plasmodial DNA has been suggested as a method of choice in endemic countries. In developing countries like Pakistan, the high cost, expensive infrastructure and laboratory personnel expertise hinders wide spread utilization of molecular assays. However, efforts should be made to implement NAT-based detection in regions with high endemicity considering low

parasitemia. As epidemiology changes, the questionnaire should be reviewed periodically.

Conclusion

Malaria appears to be very uncommon in the blood of asymptomatic male donors and screening by expensive NAT would not make economic sense. Effective donor screening using structured questionnaire to defer high risk individuals is necessitated.

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Open Access Original Article

A Systematic Review and Meta-Analysis on Prevalence of ABO and Rhesus Blood Groups in Pakistani Population

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Abstract

Objective: The main aim of this review is to document the ABO and Rh blood group distribution pattern among the population of different regions of Pakistan and the overall population of Pakistan.

Methodology: Major databases like Google Scholar, PubMed, Ovid, Web of Science, SciHub, Medscape, PakMediNet, Scopus, and Science Direct were searched. MeSH words like Prevalence, ABO, Rh, Blood Groups were used in the search engine databases. After careful review, 65 studies from January 1977 to December 2020, were included, with no gender, or ethnic restrictions.

Results: Our study recorded 160 studies of which 150 were from the database while 10 were identified through other sources (handcomb search). From these 160 studies, 40 were duplicate and were removed. The remaining 120 studies were screened properly and after the screening, additional 21 studies were excluded. Full-text articles of 99 studies were assessed for eligibility of which 34 were excluded for certain reasons. After applying the inclusion and exclusion criteria, 65 studies were included in the meta-analysis. According to the present study, the frequency distribution of the ABO blood groups in the Pakistani population was in the pattern B>O>A>AB with blood group B (34%) being the commonest, followed by O (32%), A (24%), and AB (10%). In the Rh blood group system, Rh (D) was the most prevalent antigen present in 91% of the population.

Conclusion: Blood group B is the most prevalent while blood group AB is the least prevalent in the Pakistani population. To establish a more reliable and national database of ABO and Rh blood groups in Pakistan, National Database and Registration Authority can play a pivotal role.

Keywords: Blood groups, ABO, Rhesus, Blood donors.

Introduction

The blood groups of the population are determined serologically by the presence of specific antigens on the erythrocytes. From one population to another and one region to another, the incidence of ABO and Rhesus (Rh) groups vary. ABO and Rh blood group systems are the most commonly used genetic markers and have a wide application in transfusion medicine, disease susceptibility studies, population genetics, and forensic pathology. Hence, the data of ABO and Rh blood grouping of any population set is of great importance.

The ground-breaking discovery of blood groups is attributed to the Austrian/German scientist, Dr. Karl Landsteiner, who described the first human blood group system (ABO) in 1901.[1] This landmark discovery resulted in a paradigm shift in the field of transfusion medicine.[2] Later in 1940, Dr. Landsteiner along with Dr. A.S. Weiner discovered and defined the Rhesus (Rh) blood group system.[3] The red blood cells (RBCs) or erythrocytes with A and/ or B antigens occur as the action of the glycosyltransferases enzymes that add specific sugars to the precursor substance.[4] However, a group of conformation-dependent epitopes along the Rh protein forms the D antigen.[5] On red blood cells, more than 600 surface antigens have been found[6] and numerous of these antigens that stem from one allele or are very closely related genes jointly form a blood group system.[7]At present, the International Society of Blood Transfusion (ISBT) listed 41 blood group systems representing over 300 antigens.[8] On the red cell membranes apart from the ABO and Rhesus system, many other types of antigens have been identified. Some of the important group systems are ABO, MNS, P, Rhesus, Lutheran, Kell, Lewis, Duffy, and Kidd, among

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others.[9] The discovery of blood groups significantly paved the way for safe blood and blood component transfusions around the globe with minimum adverse reactions.[10] Transfusion reactions most notably the haemolytic reaction and haemolytic disease of the new-born expresses the curative impact of the ABO and Rh blood group system depending on the ability of agglutinins of Abo and Rh blood groups systems. [11,12,13] The main goal of the present study was to assess the ABO and Rh blood group frequency pattern among the population of different regions of Pakistan and the overall population of Pakistan.

Methodology

Systematic Review and Search Strategy: The study protocol was in accordance with the PROSPERO database[14] and the main outcomes are reported following the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) guidelines.[15] We searched Google Scholar, PubMed, Ovid, Web of Science, SciHub, Medscape, PakMediNet, Scopus, and Science Direct. From January 1977 to December 2020, all the studies conducted in Pakistan were included, with no gender, or ethnic restrictions. The authors restricted the language of articles to only English. The search strategy included MeSH (Medical Subject Headings) words and keywords like 'Prevalence', 'ABO blood groups', 'Rh blood groups', and 'blood groups', and terms describing epidemiology and prevalence of blood groups. A total of 160 studies were recorded of which 150 were from the database while 10 were identified through other sources (handcomb search). The 160 articles recognized through the above-mentioned databases were presented to the Endnote version X9 software (reference manager), where 40 duplicate publications were identified and removed independently by two of the authors (IM, NS).

The remaining 120 studies were screened adequately and after the screening of title and abstract, further 21 studies were excluded. A total of 99 full-text articles were assessed for eligibility of which 34 were excluded for reasons mentioned under the below sub-heading. Review articles were excluded but their references were tracked to find any relevant article.

After applying the inclusion and exclusion criteria, a total of 65 studies on the prevalence of ABO and Rh blood groups were included in the present meta-analysis. After the selection of 65 articles that met inclusion criteria, two authors (IM, UW) reviewed all full-text articles. In 11 cases, the full-text article could not be retrieved, so corresponding authors were contacted to share their published articles. Gray literature was searched through the national and provincial health ministries' websites, annual reports, and the WHO website.

Eligibility, Inclusion, and Exclusion Criteria: Articles mentioning the geographic description of the prevalence of blood groups from the year 1977 to 2020 were included. Duplicate data, secondary data, articles published before 1977, articles with a lack of clear depiction of names of the cities, and non-availability of full-text were excluded. The focus was primarily kept on the frequencies of blood groups in the population without any affiliation to diseases, environmental or other factors, therefore, articles that showed prevalence and frequencies of blood groups concerning specific diseases and health conditions were also excluded.

Screening and Data Extraction: Each study was allotted a number for identification. The following data (based on an ad-hoc Excel spreadsheet) were later extracted from every article in the following domains: reference details (this included surname of author, title, journal, year of publication), city name, province/state name, sample size, number of and the reported prevalence and pattern of blood group in descending order (higher to lower prevalence) in the cities and then the provinces/states of the country. Any discrepancy regarding the extracted data was resolved by discussion and mutual consensus.

Article Quality Appraisal: Two authors (WU, MQ) independently judged the quality of the included articles using the JBI (Joanna Briggs Institute) quality assessment tool for prevalence and epidemiological studies with 10 questions.[16] The answer to every question was yes, no, not clear, or not relevant/applicable. The quality of each included article was scored as poor (below the mean score) and good (mean score and above).

Results

A pool of 160 records/articles was yielded after the initial search of the literature. The process of searching, retrieving, and selecting relevant studies is shown in Figure 1. Out of 160 records, 40 were duplicate articles

and were removed as such. The title and abstract of the articles were then reviewed and at this step, 21 articles were omitted. The remaining 99 articles were reviewed and 34 articles were excluded with reasons (mentioned in the methodology section) while 65 articles were selected to determine the prevalence of ABO and Rh blood groups in the country. The results of the meta-analysis of this review have been tabulated separately in the general population/blood donors, different districts/cities, and different provinces/administrative units of Pakistan.

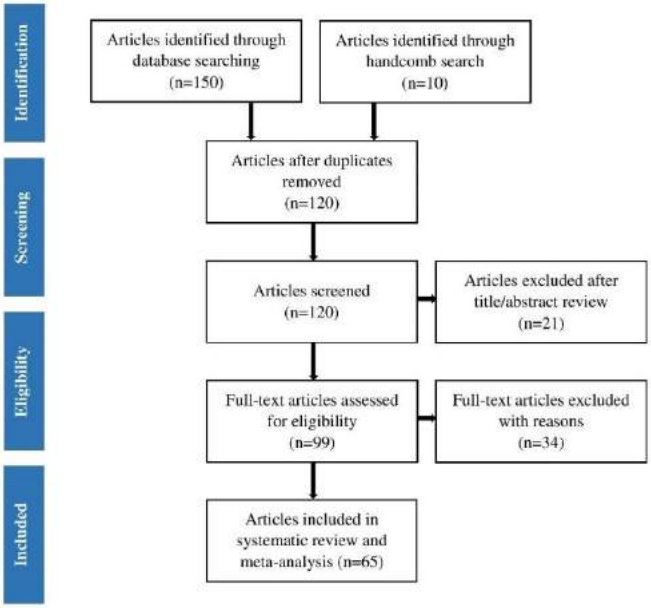

Figure 1: Flowchart of the process by which articles were identified based on PRISMA 2009 guidelines.

These results depict that in most districts/cities, the prevalence of ABO blood groups had a pattern of B>O>A>AB and O>B>A>AB except in a few studies conducted in Dir Upper[63], Gilgit[68], and Skardu[29] where it was A>B>O>AB, in Dir Lower,[53] it was A>O>B>AB, in Mirpur[43], Bannu[27], Peshawar[54], North Waziristan[62], and Karachi,[76] it was B>A>O>AB while in a study from Islamabad,[55]it was B>O, A>AB(A=O). In most districts/cities of Pakistan, the prevalence of Rh-positive blood group ranged from 88.94% to 94.83% while the Rh-negative blood group ranged from 5.17% to 11.05% except in some studies, e.g. in the study from Gilgit[68] (Rh-positive 67.33%; Rh-negative 32.66%), Wah Cant[36] (Rh-positive 73.9 %; Rh$^-$negative 26.1%), and Karachi[56] (Rh$^-$positive 97%; Rh-negative 3%).

After a complete analysis of the current study, the average pattern of ABO blood groups in Pakistan was B>O>A>AB, and Rh-positive was 91% while Rh-negative was 9%. The pooled prevalence of the studies is given in Table 2. The frequency distribution of ABO and Rh blood group systems in different provinces/administrative units of Pakistan is shown in Table 3 and Table 4, respectively.

The ABO and Rh blood groups distribution pattern in the population of different countries other than Pakistan was in the same order as O>A>B>AB except in Australia[78], Nepal[83], and Iran[89] where it was A>O>B>AB. The Rh blood groups in the population of different countries other than Pakistan was showed a similar trend, Rh-positive ranging from 92.40% to 96.81%, and Rh-negative from 3.20% to 7.60% except in Canada[81], Turkey[87], and USA[88] where Rh-positive was 83.1%, 85.9%, and 83.00%, respectively while Rh-negative was 16.9%,14.1%, and 17.00% respectively.

Discussion

The research on the ABO and Rh groups system has been of colossal interest, mainly due to its medical importance in different diseases. During the ongoing COVID-19 pandemic, the blood groups have been extensively studied to ascertain their association with SARS-CoV-2 susceptibility.[91,92] After a meta-analysis, the relative frequency of the ABO and Rh blood groups in various districts of the country was found to be nearly the same.[17,77] Similarly, the frequency distribution of ABO blood groups in different administrative units of Pakistan was almost the same and was in the order of B>O>A>AB except in Gilgit Baltistan, where it was A>B>O>AB and in Sindh, it was O>B>A>AB. The frequency distribution of Rh-positive and Rh-negative blood groups in different administrative units of Pakistan was almost the same ranging from 88.55% to 94.75% and 6.31 to 11.45%, respectively except in Gilgit Baltistan where Rh-positive was 83.98% and Rh-negative was 16.02%. This meta-analysis concluded that the average pattern of ABO blood groups in the Pakistani population was B>O>A>AB, Rh Positive was 91% and Rh-Negative was 9%. However, comparison with the data from the frequency distribution of ABO blood groups in the population of different countries other than Pakistan, i.e., UK[79], Bangladesh[80], India[82], Canada[81], Niger[84], Saudia Arabia[85], Nigeria[86], and USA[88], was in the order as O>A>B>AB, in Australia[78],

Table I: Frequency distribution of ABO and Rh blood groups in differt districts/cities of Pakistan

Population	A (%)	B (%)	AB (%)	O (%)	Rh⁻positive (%)	Rh⁻negative (%)	Pattern	Sample Size
Punjab (1977)[17]	21.20	36.16	9.05	34.14	-	-	B>O>A>AB	1,415
Karachi (1982)[18]	23.00	36.5	10.00	30.00	93.00	7.0	B>O>A>AB	3,012
Peshawar (1982)[19]	28.00	34.00	7.00	31.00	-	-	B>O>A>AB	10,049
Karachi (1988)[20]	23.5	36.5	10	30	93	7	B>O>A>AB	1,500
Swabi (1992)[21]	27.60	34.40	8.80	32.20	-	-	B>O>A>AB	3,000
Larkana (1998)[22]	25.40	32.09	9.18	33.33	-	-	O>B>A>AB	49,061
Jamshoro (1998)[22]	23.67	24.65	2.69	48.99	-	-	O>B>A>AB	49,061
Hyderabad(1998)[22]	15.69	21.79	2.99	60.15	-	-	O>B>A>AB	49,061
Karachi(1998)[22]	19.38	19.62	20.21	40.78	-	-	O>B>A>AB	49,061
Nowshera (1999)[23]	27.10	32.00	11.00	29.80	92.90	7.10	B>O>A>AB	4,510
Quetta(2001)[24]	23.2	31.7	10.1	35	94.75	5.25	O>B>A>AB	2,000
Faisalabad(2002)[25]	23.26	38.00	9.98	28.75	89.19	10.80	B>O>A>AB	1,092
Lahore (2004)[26]	22.6	32.4	8.6	30.5	93.9	6.1	B>O>A>AB	3,000
Bannu (2004)[27]	31.03	36.23	7.67	25.07	89.23	10.77	B>A>O>AB	2,581
Gujrat (2005)[28]	24.89	36.90	6.87	31.31	-	-	B>O>A>AB	2,647
Skardu (2005)[29]	30.62	26.80	15.98	26.60	94.83	5.17	A>B>O>AB	1,045
Multan (2005)[30]	21.92	36.95	7.33	33.8	92.17	7.83	B>O>A>AB	6,000
Liaquatpur (2005)[31]	20.88	35.54	2.02	44.56	90.35	9.65	O>B>A>AB	1,389
Mirpur,(2006)[32]	26.40	32.50	9.50	31.70	91.04	8.96	B>O>A>AB	3,927
Rawalpindi/ Islamabad (2006)[33]	25.53	33.32	10.04	31.09	92.45	7.54	B>O>A>AB	2,518
Mandi Bahauddin (2007)[34]	15.83	28.32	4.48	55.22	91.4	8.6	O>B>A>AB	2,542
Swat (2008)[35]	27.92	32.38	10.57	29.10	90.12	9.87	B>O>A>AB	22,897
Rawalpindi/ Islamabad (2009)[36]	22.86	33.25	10.22	31.44	90.14	9.85	B>O>A>AB	3,519
Poonch (2009)[37]	21.42	36.62	7.0	34.94	89.51	10.48	B>O>A>AB	3,328
Multan (2009)[38]	21.3	42.1	6.7	29.9	-	-	B>O>A>AB	221
Wah Cantt (2009)[36]	18	24	5	53	73.9	26.1	O>B>A>AB	4,462
Gilgit (2010)[39]	24.2	40	10	25.8	89.8	10.2	B>O>A>AB	500
Sahiwal (2011)[40]	22.00	36.85	9.84	29.30	88.94	11.05	B>O>A>AB	20,010
Lahore (2011)[41]	19.03	38.36	10.62	31.99	93.99	6.01	B>O>A>AB	1,035
Rawalpindi/ Islamabad (2012)[42]	24.2	34.3	10.1	31.3	91	9	B>O>A>AB	4,642
Mirpur (2012)[43]	25.93	32.59	17.26	24.20	83.60	14.4	B>A>O>AB	8,227
Islamabad (2013)[44]	22.54	33.58	12.47	31.39	93.44	6.55	B>O>A>AB	1,739
D.G. Khan, Muzaffargarh, Multan, Bahawalpur, Liaquatpur (2013)[45]	22.50	35.90	6.30	35.70	94.80	5.20	B>O>A>AB	60,000
Mardan (2013)[46]	22.4	34.9	10.3	32.4	94.7	5.3	B>O>A>AB	2,893
Gujranwala (2013)[47]	22.90	35.35	9.31	32.41	92.02	7.97	B>O>A>AB	4,754
Lahore (2014)[48]	24.2	37.8	9.1	28.8	93	7	B>O>A>AB	3,000
Bajaur Agency (2014)[49]	29.42	30.00	10.50	30.08	91.43	8.57	O>B>A>AB	1,200
Haripur (2014)[50]	20.75	34.20	6.51	38.52	90.16	9.83	O>B>A>AB	2,140
Lahore (2014)[51]	23.9	35.2	8.8	32.1	91.7	8.3	B>O>A>AB	1,000
Rahim Yar Khan (2015)[52]	20.97	37.41	7.65	33.95	93.64	6.35	B>O>A>AB	9,891
Dir Lower (2015)[53]	31.94	27.99	11.39	28.65	92.45	7.54	A>O>B>AB	13,758
Peshawar (2015)[54]	31.23	31.70	10.02	27.03	92.54	7.32	B>A>O>AB	429
Islamabad (2015)[55]	22	34	11	22	-	-	B>O, A> AB (A=O)	123
Karachi (2015)[56]	21	31	11	37	97	3	O>B>A>AB	100
Sialkot (2015)[57]	22.30	36.50	9.70	31.30	91.30	8.70	B>O>A>AB	1,656
Multan (2015)[58]	26.57	34.15	9.61	29.67	90.72	9.28	B>O>A>AB	937
Bahawalpur (2015)[59]	21	36	6	37	95	5	O>B>A>AB	500
Sindh (2015)[60]	25.83	28.17	8.3	37.78	95.76	4.24	O>B>A>AB	3,000
Lahore (2016)[61]	20.12	37.45	10.57	32.11	92.97	7.03	B>O>A>AB	481
North Waziristan (2016)[62]	27.4	36.7	10.9	25.0	90.6	9.4	B>A>O>AB	1,026
Dir Upper (2016)[63]	32.1	29.8	12.4	25.7	86.4	13.6	A>B>O>AB	1,000
Lahore (2016)[64]	20.38	40.45	8.09	31.06	89.48	10.51	B>O>A>AB	618
Nowshera (2016)[65]	27.38	32.02	10.80	29.77	93	7	B>O>A>AB	1,190
Faisalabad (2017)[66]	18.6	44.8	6.9	29.7	89.6	10.4	B>O>A>AB	145

Islamabad (2018)[67]	24.64	34.72	9.28	31.36	92	8	B>O>A>AB	625
Gilgit (2018)68	38.66	23.33	19.33	18.66	67.33	32.66	A>B>O>AB	1,500
Karachi (2018)69	24.00	32.02	11.24	32.72	97.22	2.78	O>B>A>AB	1,583
D.I. Khan (2018)70	24.46	36.49	8.50	30.54	90.31	9.68	B>O>A>AB	4,941
Karachi (2018)71	25.58	28.96	10.38	35.06	91.94	8.05	O>B>A>AB	385
Quetta (2018)72	19.00	37.00	12.5	31.5	-	-	B>O>A>AB	200
Karachi (2019)73	24.1	33.1	7.2	35.6	91.1	8.9	O>B>A>AB	3521
Rawalpindi (2019)74	24.76	33.91	9.51	31.82	89.03	10.97	B>O>A>AB	105,520
Karak (2020)75	22.67	31.95	15.36	30	96.26	3.73	B>O>A>AB	402
Karachi (2020)76	29.9	33.5	12.7	23.9	92.4	7.6	B>A>O>AB	394
Lahore (2020)77	22	34	10.33	33.66	94	6	B>O>A>AB	3000

Table II: Frequency distribution of blood groups (ABO and Rh) in Pakistan

Country	A (%)	B (%)	AB (%)	O (%)	Rh-positive (%)	Rh-negative (%)	ABO Pattern
Pak.	24	34	10	32	91	9	B>O>A>AB

Table III: Frequency distribution of ABO blood group system in different administrative units of Pakistan

Province/ State	A (%)	B (%)	AB (%)	O (%)	Pattern
ICT (Islamabad)	25.00	34.00	11.00	30.00	B>O>A>AB
Punjab	21.99	35.79	8.27	33.95	B>O>A>AB
KP	27.29	32.90	10.10	29.81	B>O>A>AB
Sindh	23.54	29.83	9.61	37.02	O>B>A>AB
Balochistan	21.1	34.35	11.3	33.25	B>O>A>AB
AJK	24.58	33.90	11.25	30.27	B>O>A>AB
Gilgit Baltistan	31.18	30.04	15.10	23.68	A>B>O>AB

Table IV: Frequency distribution of Rh blood group system in different administrative units of Pakistan

Province/State	Rh-positive (%)	Rh-negative (%)
ICT (Islamabad)	91.81	8.19
Punjab	91.03	8.97
KP	91.56	8.44
Sindh	93.69	6.31
Balochistan	94.75	5.25
AJK	88.55	11.45
Gilgit Baltistan	83.98	16.02

Nepal[83], and Iran[89] it was A>O>B>AB, while in Turkey[87] it was B>O>A>AB. The Rh blood groups in the population of different countries other than Pakistan, i.e. UK[79], Bangladesh[70], India[82], Nepal[83], Niger[84], Saudia Arabia[85], Nigeria[86], and Iran[89] was almost similar to our findings, Rh-positive ranging from 92.40% to 96.81% and Rh-negative from 3.2% to 7.60% except in Canada[81], Turkey[87], and USA[88] where Rh-positive was 83.1%, 85.9%, and 83.00% respectively while Rh-negative was 16.9%, 14.1%, and 17.00% respectively. It is clear from the present meta-analysis that the prevalence of ABO blood groups in Pakistan and Turkey[87] was in the same order as B>O>A>AB.

One of the reasons could be ancestral genetic makeup, however, more research is needed in this regard. Regarding the blood groups frequency distribution, the sharp difference may be attributed to terrestrial variations, environmental factors, and genetic factors.[93] In several studies carried out in different societies and cultures, for example, Bangladesh and

Table V: Comparison of ABO and Rh blood groups among different countries

Country	A (%)	B (%)	O (%)	AB (%)	Rh-positive(%)	Rh-negative (%)	Pattern
Australia[78]	38.0	10.0	49.0	3.0	-	-	A>O>B>AB
UK[79]	41.70	8.60	46.70	3.0	-	-	O>A>B>AB
Bangladesh[80]	26.6	23.2	40.6	9.6	96.81	3.20	O>A>B>AB
Canada[81]	42.00	9.0	46.0	3.0	83.1	16.9	O>A>B>AB
India[82]	23.85	29.95	39.81	6.37	94.20	5.80	O>B>A>AB
Nepal[83]	34.00	29.00	32.50	4.00	96.66	3.34	A>O>B>AB
Niger[84]	24.43	20.09	53.22	3.00	93.88	6.12	O>A>B>AB
Saudi Arabia[85]	24.00	17.00	52.00	4.0	93.00	7.00	O>A>B>AB
Nigeria[86]	21.60	21.40	54.20	2.80	95.20	4.80	O>A>B>AB
Turkey[87]	27.0	30.6	30.4	12.0	85.9	14.1	B>O>A>AB
USA[88]	40.0	11.0	45.0	4.0	83.00	17.0	O>A>B>AB
Iran[89]	45.0	11.0	40.0	4.0	92.40	7.60	A>O>B>AB
China[90]							
Pakistan (current analysis	24.00	34.00	10.00	32.00	91.00	9.00	B>O>A>AB

Latin America, racial (genetic) and environmental factors have been reported to affect the incidence of various blood groups.[94]

The evident difference between Gilgit Baltistan and the rest of the country regarding the pattern of blood groups distribution might be due to the geographical difference (high altitude) and the environment but again needs to be further studied. For varying frequency of the blood groups among the Pakistani population, different genetic and environmental factors are responsible and need to be probed further. In addition to the linkage between blood groups and blood transfusions, the ABO system is reportedly associated with cardiovascular diseases, HBV infection, preeclampsia, organ transplantation, and genetic marker of obesity, among others. Hence, the data reported in our analysis will be useful for healthcare providers, researchers, policymakers, and transfusionists. To establish a more reliable and national database of ABO and Rh blood groups in Pakistan, NADRA (National Database and Registration Authority) can play a pivotal role.

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Open Access Case Report

Unusual Presentation of Multiple Myeloma as Myeloma Ascites - Case Report

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Abstract

Multiple Myeloma is a malignant plasma cell disorder. Aggressive myeloma may involve extramedullary sites. Ascites due to peritoneal involvement by clonal plasma cells is rare at initial presentation. We report an unusual case of IgA Lambda Multiple Myeloma in a 48-year old male patient, who presented with myelomatous ascites and sparing of skeletal system.

Keywords: Multiple Myeloma, extramedullary, myelomatous ascites, plasma cells, peritoneal.

Introduction

Multiple Myeloma (MM) is a clonal neoplasm of plasma cells that constitutes about 10% of all haematological malignant disorders.[1] Multiple diagnosis of myeloma requires the presence of either 10% or more clonal plasma cells on bone marrow examination or a biopsy-proven plasmacytoma along with features of end-organ damage (hypercalcemia, renal failure, anemia, or lytic bony lesions) and three specific biomarkers: clonal bone marrow plasma cells of $\geq$ 60%, serum free light chain (FLC) ratio of $\geq$ 100 and more than one focal lesion on Magnetic Resonance Imaging (MRI)[2] Clinically, the presentation of patients may range from asymptomatic to symptomatic, with severe complications. Extramedullary myeloma is defined by the presence of plasma cells outside the bone marrow in a patient diagnosed with multiple myeloma.[3] These aggressive and poorly prognosis myelomas invade organs other than bone marrow, lymph nodes, and the reticuloendothelial system.[4] Peritoneal involvement of multiple myeloma manifesting as myelomatous ascites is a rare condition and has been described in only few case reports. [5,6] Here, we describe an unusual case of multiple myeloma with extramedullary involvement having gastrointestinal symptoms and ascites at initial presentation, with sparing of bones.

Case Report

A 48 year old male patient was admitted with complaints of early satiety, abdominal discomfort and distension after food intake associated with burps and anorexia for 1 month. There was also associated history of exertional dyspnea, generalized body weakness, undocumented weight loss, constipation and hematochezia due to hemorrhoids. His past medical history and family history were unremarkable. Physical examination revealed pallor, raised Jugular Venous Pressure, massive ascites and generalized body edema. No palpable visceromegaly or lymph node enlargement was appreciated. Initial laboratory work-up at presentation showed hemoglobin of 5 g/dL, MCV of 95 fL, white blood cell count of 6.6 × 10^9/L (granulocytes 68%, lymphocytes 15%, monocytes 11%, eosinophils 01% and metamyelocytes 05%), and platelet count of 163 × 10^9/L. ESR was 130 mm/first hour. Peripheral film showed features of mixed deficiency anemia with rouleaux formation. His serum sodium, potassium, blood urea nitrogen, creatinine, uric acid, total bilirubin, ALT, Alkaline Phosphatase and corrected calcium levels were normal at presentation. Other laboratory test results were as follows: albumin 2.59 g/dL, LDH 196 U/L, CRP 10 mg/L, Vitamin B12 127.5 pg/ml, folic acid 1.47 ng/ml, ferritin 30 ng/ml. HbsAg, Anti-HCV and anti-HIV by ELISA were negative. Upper GI endocopy was unremarkable. Colonoscopy showed internal hemorrhoids. CT-Scan

Abdomen and Pelvis revealed ascites. Echocardiography showed moderate tricuspid regurgitation, mild mitral regurgitation, moderate biatrial enlargement, dilated IVC with < 50% respiratory collapsed and ejection fraction of 60%. CT Pulmonary Angiogram and High Resolution CT were unremarkable. Bone marrow aspiration was a diluted tap while on trephine biopsy there was diffuse infiltration by plasma cells (Figure 1) with diffuse II focal III fibrosis on reticulin stain (Figure 2).

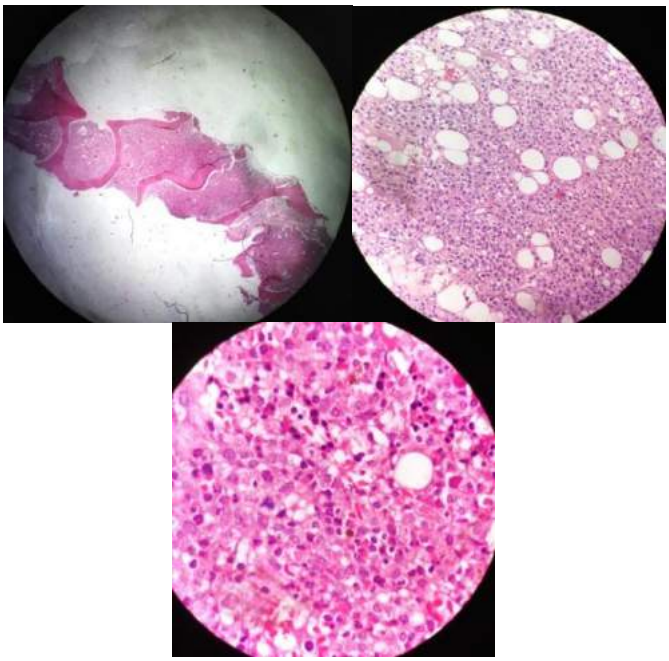

Figure 1: Bone Marrow Trephine Biopsy showing infiltration by plasma cells (H&E Stain 40X, 400X, 1000X)

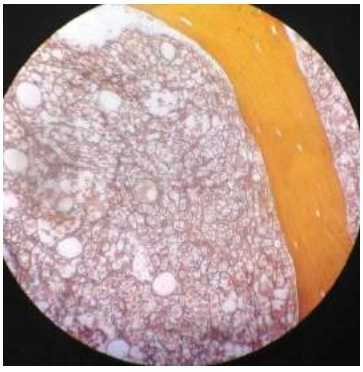

Figure 2. Bone Marrow Trephine Biopsy – showing grade diffuse II, focal III fibrosis (Reticulin Stain, 400X)

Immunohistochemical stains for kappa and lambda light chain revealed lambda monoclonality, consistent with plasma cell myeloma (Figure 3). Congo red stain for AL-amyloidosis was negative. Quantitative serum immunoglobulin levels were IgA 5499 mg/dl, IgG 437 mg/dl, and IgM 28 mg/dl. Serum protein electrophoresis showed an M band in the gamma region. Serum immunofixation showed IgA Lambda paraprotein. Bence Jones Proteins were negative. Beta-2 Microglobulin was 11.9 mg/L and no osteolytic lesions were observed on skeletal survey. Serum free light chain analysis showed free kappa of 1.6 mg/L, free lambda of 782.7 mg/L and a kappa/Lambda ratio of 0.002. This significant increase in serum free lambda light chains and a significant reduction in kappa free light chains were also indicative of lambda associated monoclonal gammopathy. The disease was given stage III according to International Staging System. Routine examination of ascitic tap showed slightly turbid, pale yellow fluid with total protein 4.48 g/dl, albumin 1.33 g/dl, LDH 110 U/L and sugar 114 mg/dl. Cytology revealed some malignant-appearing plasma cells, consistent with myelomatous involvement (Figure 4). Monoclonal band in gamma region was also confirmed on ascitic-fluid protein electrophoresis. During the work-up period, renal function tests started to worsen with reduced urine output for which alternate day sessions of Hemodialysis and Plasma exchange were carried out. Multiple units of RCC were transfused; diuretics and hematinic therapy were given.

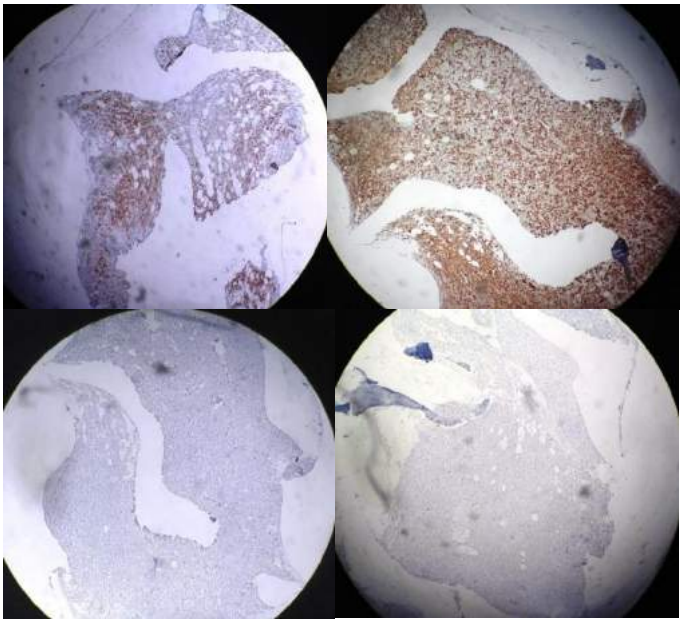

Figure 3: Immunohistochemistry on Trephine Biopsy (CD138 Positive, Lambda Positive, Kappa negative, and CD56 negative)

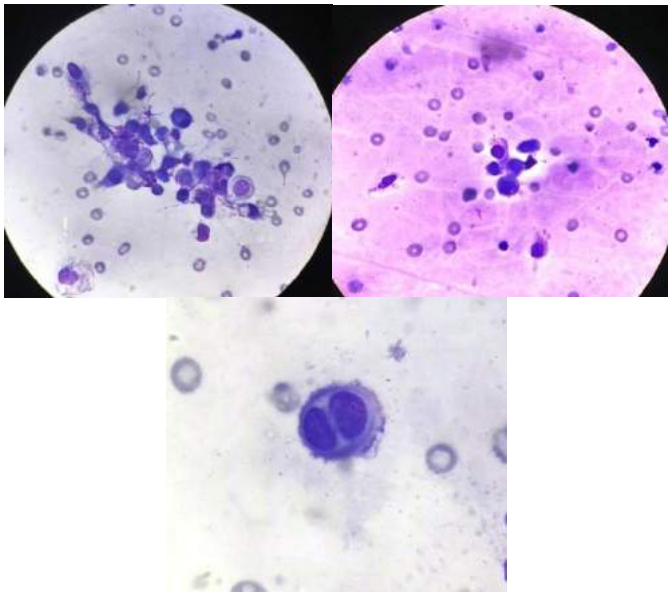

Figure 4: Ascitic Fluid microscopy showing abnormal plasma cells (Giemsa stain 400X, 400X, 1000X)

Patient was started on CyBorD (Cyclophosphamide, Bortezomib, Dexamethasone) chemotherapy regimen for myeloma (total 16 weekly doses). The patient completed the four cycles, with significant improvement in ascites, body edema, renal and cardiac function. However, before commencement of 5th cycle, the patient developed respiratory tract infection, limiting the chemotherapy initiation. His hemoglobin dropped to 7.5 g/dl, and on peripheral smear 5% plasma cells, myeloid precursors and marked Rouleaux formation were observed. Rapidly, TLC rose to 14,000/µl and the plasma cells increased to 23% (absolute count 3200/µl) with an extremely high value of serum IgA levels of >8500. Unfortunately, the patient succumbed to severe sepsis in a short time following this transformation to plasma cell leukemia.

Discussion

This rare case describes a patient with multiple myeloma who presented with gastrointestinal symptoms and myelomatous ascites. Myelomatous ascites is a rarely encountered condition and can occur anytime during the course of the disease. It is often associated with Pulmonary Hypertension due to hepatic involvement or can develop as a consequence of infectious peritonitis. Less frequently, myelomatous ascites is caused by actual involvement of peritoneum by malignant plasma cells. [7]

Several cases of myelomatous ascites have been reported in literature. It can occur as an initial sign of aggressive myeloma or develop late during its natural history.[4] Myelomatous involvement of body cavity fluids is unusual, found in less than 1% of patients, with the occurrence of pleural effusions being twice more common than peritoneal effusions, while pericardial effusions are rare. [8]

Our patient presented with myelomatous ascites due to peritoneal involvement of multiple myeloma aggravated by multiple factors such as hypoalbuminemia, renal derangement and right-sided heart failure. In contrast to secondary ascites occurring due to hepatic, renal or cardiac involvement in myeloma patients, the malignant plasmacytic ascites has large, bizzare looking, non-cohesive plasma cells, and a high cell count of 800–9,000 cells/mm^3; which can lead to diagnostic confusion with reactive mesothelial cells, atypical lymphocytes, or metastatic carcinoma cells. Immunocytochemistry, flow Cytometry or immunofluorescence can be used to differentiate the plasma cells. [9] In our patient, cell count of ascitic fluid was low with only few plasma cells, however considering the suspicion of myelomatous involvement, Protein electrophoresis was carried out revealing a monoclonal band in gamma region.

Most reported cases have been associated with IgA type of myeloma.[10] In one case series, multiple myeloma cases in which myelomatous ascites was a presenting feature, there was absolute or relative sparing of the skeleton.[6] In our case the paraprotein was IgA lambda type and there were no osteolytic lesions on skeletal survey. It is mostly associated with poor prognosis. Karp and Shareef[11] reported the median survival after the development of ascites to be only 1.5 months. However, cases of successful treatment have been described as well. In one case report, Alegre et al. showed that ascites in the course of MM may respond for longer periods to high-dose chemotherapy followed by Autologous Stem Cell Transplantation.[12] Our patient completed 4 cycles of CyBorD successfully, however he succumbed to severe sepsis and could not survive after transformation to plasma cell leukemia, in a short time period of 6 months following diagnosis.

Conclusion

In conclusion, myelomatous ascites is rare at initial presentation of multiple myeloma, has very poor prognosis and can cause diagnostic confusion.

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Open Access Case Report

Repeated Failed Attempts of Bone Marrow Aspiration in an Infant

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Abstract

Failed attempt of bone marrow aspiration can be anticipated in some cases. It can be resolved by bone marrow trephine biopsy. In an infant this occurrence of failed aspiration, even after repeated attempts can lend to a situation of not being able to make a diagnosis. In a child of less than one year age this situation can be ascribed to the site for trephine biopsy, not being mature at this age. Present case is a of similar nature where there were repeated failed attempts of marrow aspiration
Keywords: Bone Marrow Aspiration.

Case Report

A six months old male child presented with repeated chest infections, failure to thrive and progressive pallor with hepatosplenomegaly. He was febrile (102^{O} F). Respiratory rate was 65/min. Abdominal examination revealed hepatosplenomegaly. Respiratory examination revealed crepitations and ronchi. Investigations revealed leucoerythroblastic blood picture with haemoglobin with haemoglobin 7.2 g/dl,total leucocytes count 39400/mm^2 with differential count of neutrophils-30%, lymphocytes-44%,monocytes-02%, eosumphils -03%, promyelocytes-05%, myelocytes-08% and metamyelocytes-05%. Five nucleated red blood cells were seen against 100 white blood cells. Platelet's count was 50,000/mm^2. Peripheral blood smear revealed anisopoikilocytosis. Tear drop cells were seen. Reticulocytes count was 9.0%. Serum calcium level was 6.9 mg/dl. Haemoglobin electrophoresis revealed no haemoglobin disorder.

Bone marrow axamination was advised. Repeated (thrice) aspiration attempts from tibial tuberosity of both sides were failed. As per the age of child, we did not proceed for bone marrow trephine biopsy. As a result, bone marrow examination failed to give any diagnosis. In the meantime, his X-ray chest was performed due to repeated chest infections. Incidentally X-ray revealed increased bone density in ribs. Subsequently skeletal survey revealed increased bone density in different bones of body (Figures 1-4). He was diagnosed as a case of osteopetrosis (Malignant Infantile Osteopetrosis; M.I.O.P)

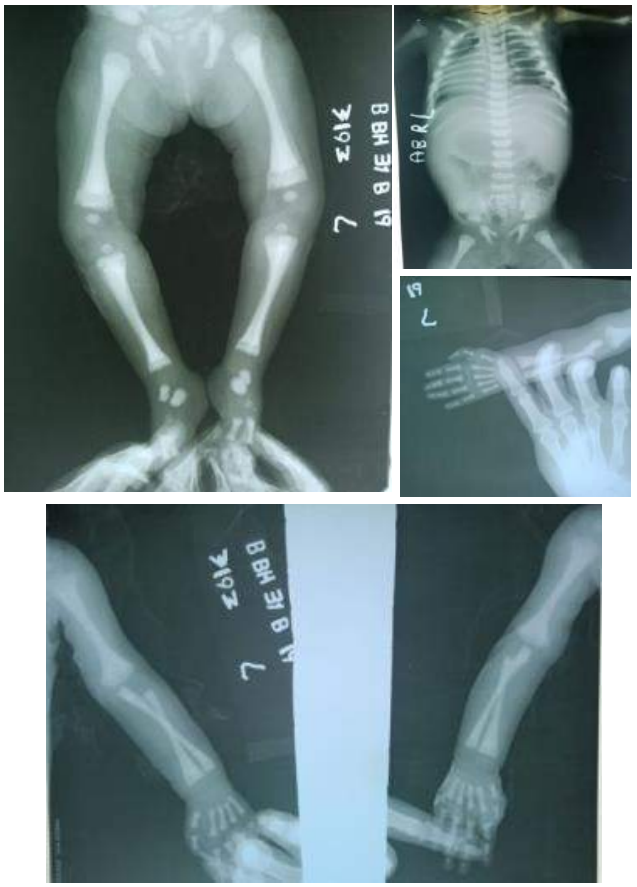

Figure 1-4. Skeletal survey report: There is an increase bone density of both humerus, radius, ulna, femur, tibia, fibula, spines and ribs. There is expansion of the visualized both femur, tibia and ribs. There is hepatosplenomegaly, which needs ultrasonographic correlation

Discussion

Osteopetrosis (O. P), also labeled as Marble Bone Disease, is a rare genetic disorder characterized by functional defect of osteoclasts resulting in failure of bone resorption, increased bone sclerosis and bone marrow failure.[1-3] The name of the disease derived from "Osteo" means bone and "petro" means stone.It may be inherited as autosomal dominant, autosomal recessive or intermediate inheritance pattern. Autosomal recessive O.P, also known as Malignant Infanatile Ostepetrosis (M.I.O.P), is the most severe form and manifests in first few months of life (Table I).[4] M.I.O.P generally starts in intrauterine life and manifests at birth or early infancy. [5] Autosomal dominant form is usually asymptomatic. It is diagnosed incidentally or may exhibit mild symptoms in late childhood or adult life, but is compatible with long term survival. [6]

Clinical presentation varies widely, based on the type of osteopetrosis and ranges in severity from asymptomatic to a fatal course. [4] Autosomal recessive MIOP is uncommon. Classic autosomal recessive MIOP is characterized by repeated infections, fractures, stunted growth, compressive neuropathies, hypocalcemia with attendant tetanic seizures, and life threatening cytopenias and characteristic radiological findings.[7] It is a severe fatal disorder. Cranial nerve entrapment neuropathies occur due to failure of the foramina in skulls to widen completely. Manifestations include deafness, visual impairment, proptosis and hydrocephalus. [8] There is gradual obliteration of marrow cavity by both bony encroachment and associated fibrosis. Although bone density is increased, the bone is more fragile than normal. [9]

Risk of developing haematological impairment in the first year of life is about 75% and its onset within three months of life is indicative of poor outcome.[2,10] Failure to thrive and increased infections because of an unexplained defect in neutrophils superoxide function are also characteristic. [11]

The abnormal expansion of bone osteoid in autosomal recessive O.P interferes with medullary haematopoiesis, resulting in life threatening pancytopenia with secondary expansion of extramedullary haematopoiesis at sites such as liver and spleen, thereby leading to hepatosplenomegaly. [7] The expanding bone can narrow nerve fragments resulting in signs of nerve compression, such as blindness, deafness, facial palsy.[4] The increase in bone density paradoxically weaken the bone, resulting in a predisposition to fractures and osteomyelitis. The longitudinal growth of bones is impaired, resulting in short stature. [7]

In present case hypocalcaemia is documented. Disturbances in calcium metabolism are well documented in O.P. In the neonatal period, children are relatively hypothyroid. In this setting normal osteoblasts function, unchecked by compensatory osteoclasts, is likely to push osteopetrotic children into hypocalcemia.[12]Hypocalcemia, hypophosphatemia and secondary hyperparathyroidism are noted in the absence of hypovitaminosis –D.[4,12,13,14]

Mutations in at least 10 genes have been

Table I: Types of Osteopetrosis

Characteristics	Infantile	Intermediate	Adult
Inheritance	Autosomal Recessive	Autosomal Recessive	Autoso-mal dominant
Genetic Basis	TCIRG, CLCN7 mutations	CLCN7, PLEKHM1 mutations	CLCN7 mutations
Diagnosis	Before age of one year	Incidentally	Incident-ally
Skeletal manifestations	Increased bone density; diffuse and focal sclerosis of varying severity;modelling defects at metaphyses; pathological fractures;osteomyelitis; dental abnormalities; tooth eruption defects; dental caries		
Haematological Manifestations	Pancytop-enia;Extramedullary haematop-oiesis; hepatospl-enomegaly	Anaemia & extramedullary haemtopoiesis	Mode-rate haema-tological failure
Neurological problems	Cranial nerves compre-ssion (II;VII; VIII)	Occasional optic nerve compression	Occasional optic nerve comp-ession
Other findings	Hydrocephalus; stunted growth; hypocal-caemia		
Onset	Perinatal	Childhood	Late child-hood or adolescence
Severity	Severe	Mild to moderate	Mild to moder-ate
Treatment	Supportive;HSCT*	Supportive	Suppo-rtive
Prognosis	Poor;fatal in infancy	Variable	Normal life expect-ancy
Recurrence risk	Parents of probond: 25% risk of recurrence in future pregnancies	Parents of probond: 25% risk of recurrence in future pregnancies	50% in future pregnancies if one parent is affected

*HSCT=Haemopoietic Stem Cell Transplantation

identified.[15] M.I.O.P involves mutation in TCRIG1 (ATPG1) encoding the a3 subunit of the vacuolar proton pump.[16,17] The primary underlying defect in all types of osteopetrosis is failure of osteoclasts to resorb bone resulting in thickened sclerotic bones, which have poor mechanical properties. It affects all bones in human body.[18] Increased bone fragility results from a failure of the collagen fibers to convert ostean properly and from defective remodeling of weven bone to compact bone.[7,2] Paradoxically increase in bone density weakens the bone, resulting in a predisposition to fractures and osteomyelitis. The longitudinal growth of bones is impaired, resulting in short stature.[7]

Defective genes in O.P are involved in the acidification machinery. Acid secretion is dependent on two key molecules, which facilitate proton transport: the proton pump vacuolar ATPas) V-ATPase) and chloride specific ion channel, chloride channel 7 (CLCN -7). Homozygous mutations in the gene encoding the a3 subunit of V-ATPase (TCIRG1) and CLCN-7 produce severe malignant O.P phenotype.[7]

Increased bone fragility results from a failure of collagen fibers to connect osteon properly and from defective remodeling of woven bone to compact bone, This leads to generalized sclerosis of bone with an increased skeletal mass. [8] Repeated infections in M.I.O.P are attributed to suppression of normal marrow haemopoiesis and unexplained defects in neutrophil superoxide function.[11]

Generalized osteopetrosis is apparent radiologically with increased bone density, often with a " bone within bone" appearance or a club like appearance. Increased bone density, diffuse and focal sclerosis of varying severity, modelling defects at metaphyses can be identified in almost all bones of the body. Pathological fractures, osteomyelitis, dental abnormalities, tooth eruption defects and dental caries are also frequent Radiographs of femur can show Erlenmeyer flask deformity. Just after birth infant may show lucent bands in distal ulna and radius Spine radiographs show sclerosis of vertebral endplates, resulting in 'sandwich vertebrae' appearance.[7].X-ray chest, in addition to bone density, may show widening at the costochondral junction.[5,15,18]

In severely affected patients the medullary cavity is filled with endochondral new bone with little remaining for haematopoietic cells.[14,19] The expanding bony trabaculae obliterate marrow, leading to bone marrow failure and a failed attempt of marrow aspiration. In an infant it can lead to a problem where it is difficult to get bone marrow trephine biopsy. On bone marrow trephine biopsy,the bony trabaculae appear thickened due to increased amount of mature lamellar bone with osteoclasts being prominent in some cases. The marrow intertrabacular spaces are occupied by connective tissue. There is loss of distinction between cortex and trabaculae. [9]

Regular ophthalmic surveillance including visual evoked potential (VEPs) is important in detecting optic nerve atrophy. Surgical decompression of the optic nerve can be employed to prevent visual loss. [20]

Pre-implantation and prenatal diagnosis is theoretically possible in families in whom the genetic mutations has been identified, thus allowing for reproductive decisions to be made. In families with severe severe autosomal recessive O.P and unknown mutations, pre-natal diagnosis may be possible using radiographs.[21] If a family decides to continue with an affected child, haematopoeitic stem cell transplantation (HSCT) before the age of three months can be planned with the aim of improving neurological outcomes. Once neurological complications emerge they cannot be reversed even after HSCT. [7]

At present, other than HSCT, at large treatment is supportive. Fractures and arthritis are common and require meticulous treatment due to brittleness of the bone and relatively frequent occurrence of secondary complications such as delayed union or non-union of fractures and osteomyelitis.[22]

Bone marrow transplantation (BMT) or Hemopoietic stem cell transplantation (HSCT) is the only curative option. It is better to contemplate HSCT before the emergence of complications, as it cannot reverse neurological complications. The best time is within one year of birth, more preferably within first few months of life. Recipients of HLA identical BMT or HSCT have been reported to have five years survival of 73 to 79%.[12,23-25]

Interferon gamma (IFN γ Ib) treatment has been reported to result in improvement in immune function, increased in bone resorption and increased in bone

marrow space.[26,27] Inteferon gamma therapy has been found to decrease the rate of infections and transfusion requirements after 24 months of therapy.[24]

Ongoing research into osteoclast physiology is likely to result in novel therapeutic targets. For example, low levels of bone resorption are observed in even severely affected patients, pointing to the presence of multiple acidification mechanisms. The activation of alternate acidification including he Na+/H+ antiporters have been proposed as therapeutic targets.[28]

Bubshalt DK et al reported a case of M.I.O.P who presented at the age of two months with a history of recurrent fever, recurrent pneumonia, developmental delay and infantile spasms . Upon exmiantion found to have hepatosplenomegaly, axial hypotonia, limb spasticity and visual impariement.[5] Local data search revealed pool apart spectrum of O.P, justifying inheritance pattern. Asif N et al (2012) documented an infant of M.I.O.P with widespread skeletal changes, hepatosplenomegaly and marrow failure. [2] Ahmed S (2008) reported an adult soldier whose X-rays incidentally revealed end plate sclerosis of thoracic vertebral bodies (Rugger-Jersey- Spine). He did not show any skeletal, haematological and neurological findings which can be ascribed to sclerosis. [8] In MIOP prompt diagnosis and earliest bone marrow or HSCT (transplant within six months of age) ensures cure and circumvents visual, auditory and other defects which are not likely to be recovered if transplant is delayed. Prenatal molecular and/or radiological screening needs exploration.[12,25,29] Repeated bone marrow aspiration failure in an infant called for to rule out a rare occurrence of osteopetrosis, as is evident from this case report.

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