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Original Article

Determination of Etiological Factors associated with Aplastic Anaemia in Rahim Yar Khan District of Southern Punjab

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Objective: To determine the clinico-haematological profile and etiological factors of aplastic anaemia in patients under study.

Material & Methods: This cross sectional study was conducted on 34 aplastic anaemic patients in the department of Department of Haematology and Blood Transfusion, Sheikh Zayed Medical College/Hospital, Rahim Yar Khan with cases referred from medicine, pediatric and out patients departments over a period of 22 months from October 2008 to July 2010. Patients of all ages and sex were included in the study. Data was entered into SPSS version 15 and analyzed descriptively.

Results: Results depict that most of the patients were in the age range 5-35 years with mean± SD (22.18±9.107 years). 82.35% patients were males and 17.65% were females. Out of 34 cases diagnosed as aplastic anaemia, 30 cases (88.2%) were suffering from acquired aplastic anemia while 4 (11.7%) cases were found to have inherited aplastic anaemia (Fanconi Anaemia). Out of 34 patients with acquired aplastic anemia, 23 (67.6%) patients had drug induced marrow hypoplasia with major marrow suppressant effect produced by chloramphenicol and sulfa formulated drugs in 14 (60.87%). 9 (39.13%) patients used non-steroidal anti-inflammatory drugs particularly Indomethacin and heavy metals incorporated in herbal medicines. Only 4 cases (11.8%) were diagnosed as inherited aplastic anaemia with major clinical manifestations in the form of hyperpigmentation of skin, short stature and hypoplastic thenar eminences. Interestingly there was not a single patient with positive viral serological profile (e.g HCV, HBsAg, HIV etc).

Conclusion: Majority of the studied patients had acquired aplastic anaemia. Antibiotics particularly Sulfonamides & Chloramphenicol, non steroidal anti-inflammatory drugs, gold salts and non-specified heavy metals are important causes of bone marrow suppression.

Keywords: Aplastic Anaemia, Etiological factors, Bone marrow suppression

Introduction

Aplastic anaemia results from inability of bone marrow to effectively produce blood cells. This leads to peripheral single cell cytopenias such as pure red cell aplasia or amegakaryocytic thrombocytopenia with primary defect occurring in committed cell lines whereas defect in the pleuripotent stem cells leads to aplastic anaemia involving granulocytic, erythroid and megakaryocytic cell lines. The remaining cells in the marrow are morphologically normal. Minor dyserythropoietic changes may be observed in the bone marrow reflecting marrow stress due to concomitant folic acid or vitamin B12 deficiencies, manifesting as macrocytosis of red blood cells in the peripheral blood and megaloblasts in the bone marrow.[1] Overlap between these two groups may be observed with single cell cytopenia transforming into multi-lineage cytopenias / pancytopenias and vice versa. Fibrosis of marrow is not a feature of aplastic anaemia distinguishing it from myelodysplastic syndrome or myeloproliferative disorders.[2] Broadly, aplastic anaemia is classified into acquired aplastic anaemia[1] and Inherited aplastic anaemia.[2] It is being observed that acquired aplastic anaemia becomes inevitable after exposure to ionizing radiation and cytototoxic drugs. This effect is usually dose dependant. The disease is infrequent during the first year of life, with a progressively rising incidence until the age of 20. A plateau is observed between ages 20 and 60 years, followed by an increase after the age of 60. Genetic predisposition is also found in some families.[3] Fanconi in 1926 described an important constitutional/genetic condition which is transmitted as autosomal recessive pattern and associated with progressive marrow failure. This entity was later named as Fanconi anaemia The effected individuals usually have one or more somatic abnormalities manifesting as short stature, hyperpigmentation of skin, microcephaly, microstomia, cryptorchidism or abnormalities of kidney (horse shoe shaped kidney). They also have increased risk of acute myeloid leukaemia or squamous cell carcinoma.[4] The present

study was undertaken to evaluate the clinico-haematologic profile and etiologic factors of aplastic anaemia.

Material and Methods

This study was conducted in the Department of Haematology and Blood Transfusion, Sheikh Zayed Medical College/Hospital, Rahim Yar Khan over a period of 22 months from Oct 2008 to July 2010. Thirty four patients were included. The inclusion criteria were Hb <10 gm/dl, RBC count <3.0 $x10^{12}$/L, Absolute Neutrophil Count <1.5 $x10^{9}$/L, Platelet count <100 $x10^{9}$/L and bone marrow cellularity 25% or <25%. These patients did not receive chemotherapy or radiotherapy and thus iatrogenic bone marrow aplasia/ hypoplasia was excluded from the study. Socioeconomic status, history of exposure to drugs, occupation of adult patients and parents of paediatric patients were noted. General physical and systemic examinations were carried out on all patients. Complete blood picture including peripheral blood film, reticulocyte count, LAP score, fetal Hb, bone marrow aspiration/bone marrow trephine biopsy, Hams test and viral profile for hepatitis B, C and HIV were performed in all 34 cases.

In addition, cytogenetic analysis on peripheral blood and bone marrow lymphocytes using RPMI 1640 culture medium, Hanks Balanced Salt Solution, Phytohaemagglutinin (PHA) and trypsin was performed in patients suffering from Fanconi anaemia. Mitomycin C was added to the culture medium to induce chromosomal breaks in the form of single chromatids, isochromatids and accentric fragments.

These exaggerated changes to mitomycin C were then compared with healthy control. All 34 patients were also subjected to serological screening for Hepatitis B, C and HIV virus by ELISA method.

In cases of acquired aplastic anaemia, socioeconomic status, occupation of patients/ parents of pediatric patients and exposure to drugs particularly chloramphenicol, sulphonamides, non steroidal anti-inflammatory drugs (particularly indomethacin), anticonvulsant drugs, antidepressant drugs, antithyroid drugs and herbal medicines were evaluated.

For statistical analysis, means and standard deviations were calculated for quantitative variables (e.g age, Hb, WBC, ANC, RBC and platelets), while count and percentages were computed for qualitative variables (e.g gender, clinical features etc.)

Results

Results showed that mean of patients' age was 22.18 yrs with ±SD (9.107), ranges (11-35 years & 5-10 in acquired and inherited groups, respectively) Male to female ratio was 4.6:1. Based upon history, clinical presentation, laboratory parameters and cytogenetic analysis, these were further subdivided into 30 cases as suffering from acquired aplastic anaemia (88.2%) and 4 as inherited aplastic anaemia (11.7%). Majority of patients, 23 (76.6%) in acquired aplastic anaemia were adult with age range from 17 to 35 years, while only 7 (23.3%) patients were in the pediatric age group with age range from 11 to 15 years (13.1±2.04 years). All 4 patients diagnosed as inherited aplastic anaemia (Fanconi anaemia) were in the pediatric age group. Sex distribution showed that about 82.35% patients were male and other 17.65% were females.

The most common clinical presentation was pallor which was seen in all 34 cases (100%). Fever was present in 29 cases (85.2%) and bleeding diathesis in 23 cases (67.6%). Most common manifestation was bleeding from the gums and nose which was seen in 17 cases (50%) followed by bruises/ecchymosis in 4 cases (11.7%). In addition, bleeding from GIT was seen in 2 cases (5.8%) of acquired aplastic anaemia.[5] Presentation with short stature and hyperpigmentation of skin was seen in 3 cases (8.8%) of inherited aplastic anaemia. Hypoplastic thenar eminence was seen in only 1 case (2.9%) of inherited aplastic anaemia.[6] Clinical profile is shown in **table 1.**

Bone marrow trephine biopsy revealed markedly hypocelllar marrow (<10% cellularity) in 18 cases of acquired aplastic anaemia (60.0%) and 1 case of inherited aplastic anaemia (25 %). Hypocellularity was found to be moderate (25%) in rest of 15 cases of aplastic anaemia including both acquired and inherited types. Comparative descriptive analysis of different quantitative parameters in both groups (acquired & inherited) is shown in **table 2.**

Cytogenetic study was performed in four cases presenting with short stature, hyperpigmentation of skin and hypoplastic thenar eminences utilizing peripheral blood and bone marrow aspirate. In 3 cases of Fanconi anaemia, the study showed non specific chromosomal changes in the form of random chromosomal breaks, presence of isochromatid material and chromatin exchange. These changes reflected fragility of chromatin material with excessive response to mitomycin C. In the fourth case no change could be identified.[7]

Two patients (5.8%) in the adult age group presenting with acquired aplastic anaemia were found to have

positive Ham Test. Bone marrow aspiration and trephine biopsy showed relatively increased number of normoblasts in hypoplastic fragments. Leucocyte Alkaline Phosphatase staining on peripheral blood neutrophils (LAP/NAP score) was decreased to <50 with parallel control blood sample showing 150-200.These patients were considered as transitional cases between aplastic anaemia and paroxysmal nocturnal haemoglobinuria.[8] A close relationship was seen between socioeconomic status and the incidence of acquired aplastic anaemia. Twenty five patients (73.5%) belonged to a lower socioeconomic class with an average per annum family income of Rs 91,000. Unethical aspect of medical practice was also found as a major contributory factor to the development of bone marrow aplasia. This observation was supported by positive drug history in 23 cases (67.6%) of acquired aplastic anaemia. Majority had a history of intake of chloramphenicol and sulfa formulated drugs. This was seen in 14 cases (60.8%) of drug induced acquired aplastic anaemia. In addition positive history of intake of non-steroidal anti-inflammatory drugs particularly indomethacin and herbal medicines containing non-specified heavy metals was found in 9 cases (39.1%). These drugs were found to produce significant degree of myelosupression both through idiosyncratic and drug cumulative effect and can be considered as predominant etiological factors in our prospective study. The detrimental role of these drugs in bone marrow suppression is also observed in other international studies.[9,10] In the remaining 7 patients with acquired aplasia no definite cause could be identified, most probably attributable to negative viral serological profile for Hepatitis B, C and HIV and negative specific drug intake history.

Table-2: Clinical presentation in aplastic anaemia.

Clinical features	Acquired aplastic anaemia n(%)	Inherited aplastic anaemia n(%)
Anaemia	30 (88.2)	4 (11.7)
Fever	27 (79.4)	2 (5.8)
Bleeding Gums/Epistaxis	16 (47.0)	1 (2.9)
Bruises/Ecchymosis	3 (8.8)	1 (2.9)
GIT Bleeding	2 (5.8)	-
Short stature/Hyper pigmentation of skin	-	3 (8.8)
Hypoplastic thenar eminence	-	1 (2.9)

Discussion

Aplastic anaemia implies pancytopenia in the peripheral blood and a hypocellular marrow with specific failure of the bone marrow precursor cells to produce mature haemopoietic cells. The hypocellular marrow becomes progressively replaced by fat cells. Few cells produced by the marrow are morphologically normal with normal life span. In addition these mature haemopoietic cells are not abnormally sequestered in the spleen nor they are subjected to abnormal environment in the peripheral blood. Reducton of at least two out of three peripheral

Table-2: Descriptive Analysis of Quantitative Parameters

		Age (Years)	Hb (g/dL)	WBC ($\times10^9$/L)	ANC ($\times10^9$/L)	RBC ($\times10^{12}$/L)	Platelets ($\times10^9$/L)
Acquired Aplastic	Mean±SD	24.10±7.832	5.210±1.26	2.063±798	.815±504	2.02±664	18.14±7.012
Anaemia (n=30)	N	30	30	30	30	30	30
	Minimum	11	3.0	1.0	.2	1	8
	Maximum	35	7.0	3.6	2.9	3	32
	Range	24	4.0	2.6	2.7	2	24
	Std. Error of Mean	1.430	. 2301	.1456	.0920	.121	1.280
Inherited Aplastic	Mean±SD	7.75±2.217	5.463±1.54	2.625±1.144	.850±40.41	2.42±779	18.55±9.28
Anaemia (Fanconi	Minimum	5	3.4	1.2	.3	2	11
Anaemia) (n=4)	Maximum	10	6.3	3.6	1.2	3	30
	Range	5	3.3	2.4	.9	1	19
	Std. Error of Mean	1.109	.7734	.5721	.2021	.239	4.641

aplastic anaemia (1) haemoglobin < 10gm/dl (2) platelets < 100 x 10^9/L and (3) absolute neutrophil count 1.5 x 10^9/l or < 1.5 x 10^9/L.[11] Aplastic anaemia is classified into acquired and inherited types based upon clinical presentation, duration, etiological factors and cytogenetic changes. Major etiological factors in acquired aplastic anaemia are either direct effect of ionizing radiation on the bone marrow or cytotoxic effect of drugs used in malignant conditions. Indirectly non-cytotoxic drug induced marrow aplasia/ hypoplasia involve idiosyncratic and immune mechanisms. This is especially seen with intake of sulphonamides, chloramphenicol, non-steroidal anti-inflammatory drugs, gold salts, phenytoin, carbamazepine, carbimazole, thiouracil, phenothiazine, chlorpropamide, chloroquine and non-specified heavy metals which constitute important ingredients of herbal medicines.[12,13,14] In our present study considerable proportion of patients gave positive history of intake of above mentioned medicines from registered general medical practioners and registered/non-registered practioners of herbal medicines.. In particular both dose dependent and idiosyncratic mode of bone marrow suppression by chloramphenicol is observed involving single dose or multiple doses. The effect is mediated through the action of drug on mitochondrial DNA.[15] Development of aplastic anaemia following the use of co-trimoxazole (combination of trimethoprim and suphonamide moiety) and anti-malarial drug (combination of pyrimethamine and sulphonamide moiety) is also seen with mechanism of marrow suppression mediated through idiosyncratic and dose cumulative effect. This is particularly true for sulphonamides and related sulfa drugs. However trimethoprim (component of co-trimoxazole) was also found to produce significant degree of neutropenia involving the same mechanism mentioned above. In our present study, drug induced myelosuppression was seen in fourteen adult and paediatric patients. Link between the indiscriminate use of indomethacin(non-steroidal anti-inflammatory drug), gold salts prescribed by general medical practitioners for rheumatoid arthritis and osteoarthritis and herbal medicines containing non-specified heavy metals with bone marrow suppression was documented in nine patients. This observation was found to be in parallel with international studies.[16] Chemicals specially benzene present in petroleum products are also incriminated as causes of myelosuppression. The magnitude of suppression is directly proportional to the quantity exposed .[17,18] Established link with benzene toxicity however could not be identified in our study. This may be attributed to inadequate occupation history and lack of regular followup of patients. Pesticides and chemicals used in agriculture and wood furniture industry have been linked to the development of bone marrow aplasia. In our study only two cases who were farmer and carpenter by profession respectively reported with pancytopenia and bone marrow hypocellularity, most probably the result of deleterious effect of these chemicals on bone marrow function. Immune mechanisms mediated by drugs (antithyroids, anticonvulsants antidepressants), viruses (hepatitis A ,B, C, Epstein-Barr virus, HIV) and autoimmune diseases are also incriminated as causes of acquired aplastic anaemia.[19,20,21] However due to absence of specific drug intake history and negative serological profile in our patients, significance of these etiological factors as bone marrow suppressants was not considered.

Pathogenesis of aplastic anaemia is still not fully established. However immune mechanism is being focused upon as shown by the clinical response to anti-lymphocyte globulin. The cytotoxic T lymphocytes release interferon-gamma and tumor necrosis factor-alfa that are inhibitory to haemopoietic stem cells.[22] Tumor necrosis factor-alfa also promotes Fas antigen expression on haemopoietic stem cells (CD 34 cells) leading to their enhanced apoptosis and reduced survival. Genetic predisposition in the form of association of HLA-DR2 with acquired aplastic anaemia is also seen in some cases of acquired aplastic anaemia.[23]

Fanconi anaemia, is an important inherited aplastic anaemia. It is transmitted as autosomal recessive pattern. In our study only four cases were seen with variable clinical manifestations. The mean age was 7.75 years. Patients with Fanconi anaemia usually present with more marked fall in Hb and platelets as compared to granulocytes which are preserved in the early stages. The bone marrow also shows increased haemophagocytosis in addition to hypocellularity. These changes can be compared to fat replacement of hypocellular marrow in acquired aplastic anaemia with minimal or no haemophagocytosis.[24] Patients with Fanconi anaemia also have an increased risk of acute myeloid leukaemia and squamous cell carcinoma as documented in other international studies.[25,26] However such transformation still requires to be established in our study with continuous follow up of patients. Increased fragility of chromatin material with exaggerated non specific chromosomal

breaks to mitomycin C was also seen in our patients suffering from Fanconi anaemia.[27,28,29]

Conclusion

Majority of the studied patients had acquired aplastic anaemia. Antibiotics particularly Sulfonamides & Chloramphenicol, non steroidal anti-inflammatory drugs, gold salts and non-specified heavy metals are important causes of bone marrow suppression. Unethical approach by registered general medical practitioners and non registered practitioners in herbal medicine be strongly discouraged with a view to limit the unjudicious use of anti-inflammatory drugs, anti-malarial drugs and antibiotics such as chloramphenicol and sulphonamide which are known to produce suppressant effect on bone marrow haematopoitic function.

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