

Original Research Article

Etiopathogenesis of aplastic anemia in children: a case control study

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ABSTRACT

Background: Aplastic anemia is not an infrequent clinical syndrome that we encounter. In about two thirds of cases of aplastic anemia it is not possible to identify any likely cause. Aim of the study was to study the role of organochlorine compounds, Parvovirus B19, Hepatitis viruses B and C and HIV I and II in causation of aplastic anemia in children.

Methods: 25 children of bone marrow biopsy proved aplastic anemia and 25 age Matched controls were investigated for the presence of Parvovirus B19, Hepatitis viruses B and C, HIV I and II and for increased levels of organochlorines in blood and bone marrow. ELISA technique to detect antibodies against Parvovirus B19 (IgM), HCV (IgG), HbsAg, HIV I and II was used. Gas chromatography was used to measure blood levels of organo-chlorine compounds α , β , γ , δ HCH, p-p DDE.

Results: out of 25 children of aplastic anemia 5 cases (20%) were IgM ELISA positive against Parvovirus B19, 6 cases (24%) were positive for IgG antibody against HCV and 1 case (4%) was Australia antigen positive. 14 cases (56%) showed increased levels ($>\text{mean} \pm 2\text{SD}$) of organochlorine compounds α , β , γ , δ HCH, p-p DDE. None of the cases were positive for HIV I and II. None of the controls were positive for Parvovirus B19 (IgM) neither for HCV (IgG). Multiple factors (>1) were positive in 4 cases (16%). 5 cases (20%) didn't have any positivity for studied factors. 22 cases (88%) of aplastic anemia children were >5 years of age. 21 cases (84%) belonged to rural areas. 11 cases (44%) presented in the month of March and April. Parvovirus B19 was more prevalent (80% cases) in the older age group of children (8-12years).

Conclusions: Majority of virological agents contribute to non-severe aplastic anemia. Significant association was found between very severe and severe aplastic anemia with organochlorine compounds. However larger community based studies are needed to correlate this.

Keywords: Aplastic, ELISA, HCV, HCH, Hepatitis

INTRODUCTION

Aplastic anemia is not an infrequent clinical syndrome that we encounter. In about two thirds of cases of aplastic anemia it is not possible to identify any likely cause.

Amongst rest, drugs, viruses, environmental toxins and auto-immune disorders may be identified as probable cause.¹ Chloramphenicol, Phenyl butazone, Sulfonamides, Salzopyrine -Piroxicam and Gold salts are leading drugs associated with aplastic anemia. Parvo

virus B19, hepatitis and flaviviruses, Cytomegalovirus, Epstein Barr virus and HIV viruses are among the virological agents.² Immune diseases such as eosinophilic fasciitis, hypogammaglobulinemia, thymoma, pregnancy, and paroxysmal nocturnal hemoglobinuria are the other well-known etiologies associated with acquired group of aplastic anemia. A number of environmental factors have also been implicated as risk factors for aplastic anemia.^{3,4}

Pesticides may be among the factors etiologically related to aplastic anemia. Lipophilic pesticides are commonly found in human body and can lead to disruption of microenvironment in bone marrow as well as proliferation of stem cells. Present study was aimed to identify those factors with available resources that play role in etiopathogenesis of aplastic anemia.

METHODS

It was a case control study, conducted in the department of Pediatrics, Gandhi memorial and Associated Hospitals, King George’s Medical University, Lucknow, a tertiary care centre. Children of <12 years of age, presenting in the Pediatrics indoor and outdoor are included.

25 children who presented with pancytopenia and diagnosed on the basis of bone marrow biopsy with aplastic anemia, were included and investigated.

A questionnaire was prepared to identify the risk factors exposure like, use of pesticides in the fields, any accidental chemical exposure and exposure to drugs, history suggestive of HIV, hepatitis and Parvovirus B19 infection. Complete clinical examination was done. To

detect anti Parvovirus B19 IgM antibodies levels by ELISA, Parvovirus B19 nucleic acid by PCR, none marrow aspirates and blood samples were drawn. ELISA technique was used for detection anti HIV I and anti HIV2 antibodies and HBsAg in serum. Zhongshan HCV kit by ELISA method was used for detection of anti HCV antibodies. Gas chromatography was used for estimation of persistent blood levels of α , β , γ , δ HCH, p-p DDE pesticides.

Controls: age and exposure matched (those children of approximate same age group living in the same environment mostly these elder or younger brother or sisters) 10 controls were taken for pesticides and 30 controls for parvovirus B19.

RESULTS

A total of 25 cases of Aplastic or Hypoplastic anemia were enrolled over the study period of August, 2003 to July, 2004. More than half of the cases of Aplastic anemia were of non-severe category. Parvovirus B19 was the probable etiological agent in 20% of Aplastic anemia.

Table 1: Distribution of pesticides, HBV, HCV, parvo virus B19 and HIV among aplastic anemia cases.

Various factors	Number	Percentage
Parvo virus B19	5	20
HCV	6	24
HIV I and II	0	0
HbsAg	1	4
Pesticides level (>mean+2SD)	14	56
Multiple factors positive	4	16
None	5	20

Table 2: Association of various factors and severity of aplastic anemia cases.

Parameter	HBsAg positive (n=1)	Anti HCV positive (n=6)	Parvo virus B19 positive (n=5)	Pesticides (n=14)
VSAA	0	0	0	3 (21.4%)
SAA	0	0	1 (20%)	3 (21.4%)
Non-severe	1 (100%)	6 (100%)	4 (80%)	8 (57.2%)

Table 3: Comparison of various blood pesticides levels among aplastic anemia cases and controls.

Pesticides	Cases (n=25) (Mean±SE)	Controls (n=10) (Mean ± SE)	P value
α HCH (n=7)	8.108±0.25	4.126±0.16	0.0094
γ HCH (n=13)	16.900±0.67	3.601±0.41	0.0044
β HCH (n=0)	6.873±0.42	16.459±2.77	0.1984
δ HCH (n=7)	4.593±0.27	0.702±0.12	0.0876
p-p DDE (n=7)	29.678±1.40	11.987±0.99	0.1343

Majority of the virological agents (Parvovirus B19, HCV and HBV) contribute to non-severe aplastic anemia.

Parvovirus B19 is more prevalent in the older age group of children (8-12 years). A significant association was

found between very severe aplastic anemia and severe aplastic anemia with chemical and pesticides exposure

and high blood pesticide levels. No significant drug exposure was found in any of the case.

Table 4: Comparison of severity of anemia and those who had significantly elevated pesticide (mean \pm 2SD) levels.

Pesticide	VSAA	SAA	NSAA	Total
α HCH	2 (28.6%)	2 (28.6%)	3 (42.8%)	7
γ HCH	2 (15.4%)	3 (23%)	8 (61.6%)	13
β HCH	0	0	0	0
δ HCH	1 (14.3%)	2 (28.6%)	4 (57.1%)	7
p-p DDE	1 (14.3%)	1 (14.3%)	5 (71.4%)	7

α HCH = α -Hexachlorocyclohexane, γ HCH = γ -Hexachlorocyclohexane, β HCH = β -Hexachlorocyclohexane, δ HCH = δ -Hexachlorocyclohexane, p-p DDE = 1,1-Dichloro-2,2-bis (p-chlorophenyl) ethylene).

DISCUSSION

Aplastic anemia, a disorder of bone marrow malfunction, is of multifactorial in origin and is much less prevalent below 5 years of age except for congenital cases. The theoretical basis for marrow failure includes primary defects in or damage to the stem cell or the marrow micro environment and their interplay with immunity.⁶⁻⁹ Autoimmune basis for aplastic anemia has also been suggested, as in these patients the stromal cell function is normal, including growth factor which is implicit in the success of marrow transplantation as frequently the stromal elements remain of host origin.^{5,6} Hepatitis associated aplastic anemia was also found in few of the studies by autoantibody production and by T cell dysregulations.^{3,10-12} In this study total 7 cases were positive for hepatitis B and C, but all of them had history of blood transfusions, so these findings were ignored. 84% of aplastic anemia cases in this study were from the community belonging to rural areas. Protein energy malnutrition did not seem to change any scenario in aplastic anemia cases presentation. 44% of the aplastic anemia cases presented in months of March and April. 56% of cases of aplastic anemia had total duration of illness of less than one month. These facts support the theory of pesticides induced aplastic anemia as pesticides are more commonly used in rural areas commonly used at crops cultivation.

Organo-chlorine compounds are fat soluble and fairly well concentrated in the bone marrow and disrupt the microenvironment essential for normal erythropoiesis. Lindane i.e. HCH is well known to do so. The severe and very severe aplastic anemia cases in this study also matched the picture with those who develop after severe pesticides exposure.^{22,23}

A recent study from china found six cases of severe aplastic anemia associated with active or recent parvovirus B 19 infection from 30 studied cases. Comparably, in our study too, 5 cases (20% of all

aplastic anemia cases) were positive for IgM antibodies as well as for DNA PCR for parvovirus B 19 virus. This virus should therefore be considered as an important possible etiologic agent in some children with aplastic anemia. Two hypotheses can be advanced to understand the role of parvovirus B19 infection in the etiopathogenesis of aplastic anemia, the first of which involves the direct effect of parvovirus B19, as it has been shown that the cellular receptor for this virus is an antigen of the group blood P, which is present not only on erythrocytes and erythroblasts, but also on the megakaryocytes and fetal liver cells.¹⁵ Therefore parvovirus B19 infection can result in transient aplastic crises, congenital or acquired pure red cell aplasia and also idiopathic thrombocytopenic purpura.¹³⁻²⁰

The second hypothesis is based on immunological mediation. In virus associated haemophagocytic syndrome with acute parvo B19 infection, raised cytokines such as interferon γ would impair regulation of the phagocyte system, resulting in pancytopenias and/or decreased haematopoiesis.^{19,20} The recovery of hematopoietic function after immunosuppressive therapy is the strongest argument for this hypothesis. Pesticides and parvo B19 virus are definitely related to aplastic anemia cases. However larger community based studies are needed to correlate these findings. A direct association could not be established with other virological agents.

CONCLUSION

Aplastic anemia is multi-factorial in origin and Parvo B19 and pesticides both contribute to pathogenesis of the aplastic anemia but of different severity levels. Majority of virological agents contribute to non-severe aplastic anemia.

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