Asian Journal of Medical Sciences 2(5): 227-232, 2010
ISSN: 2040-8773

Submitted Date: August 19, 2010 Accepted Date: September 17, 2010 Published Date: November 10, 2010

Chromosomal Breakage Study in Aplastic Anemia Patients in India

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Abstract: The disease management of aplastic anemia patients is, to an extent, based on the etiology i.e., constitutional or acquired. Chromosomal breakage study using Mitomycin-C is widely used for this differential diagnosis in India. The present study was undertaken to find out the frequency of constitutional aplastic anemia. This prospective study was carried out at Immunology and Molecular biology Lab of Apollo hospital during July, 2007 to June, 2009. Clinico-hematologically classified 300 aplastic anemic patients that have been catered to the hospital for their differential diagnosis of aplastic anemia and their respective age and sex matched healthy controls were processed for chromosomal breakage study. Patient's habitat, clinical symptoms, differential blood count and history of drug exposure and post viral development of the disease were documented. The survival rate was documented after 2 years of diagnosis. Relative risk was estimated by odds ratio (OR) with 95% confidence interval (CI) in matched cases and controls. A significant increase of chromosomal breakages seen in 9.40% patients. 8 of 83 (9.64%) patients of > 21 yrs of age and 19 of 204 (9.31%) of ≤21 years of age showed increased breaks. The sex ratio was 3.2:1. Moderate, severe and very-severe levels of disease were seen in 27.6, 69.8 and 2.6% of the patients respectively. The survival data documented for 100 patients suggest 60% mortality. 9.40% had evidence of constitutional aplastic anemia in contrast to previously published data from India where the proportion ranges from 11-42%. The skewed sex ratio in our study probably reflects the gender bias in our society. No significant difference ($p<0.932$) was seen in proportion of inherited disease in both ≤21 (71.1%) yrs and > 21 yrs age groups (28.9%). Patients with constitutional disease could not be differentiated on the basis severity. The high mortality rate raise a need to analyze these patients on a molecular platform to dig out the genetic factors involved, if any.

Key words: Aplastic anemia, chromosomal breakage studies, mitomycin-c, indian scenario, chromosomal anomalies, fanconis anemia

INTRODUCTION

In last few decades, India has experienced the improvements in the nutritional and health infrastructure, social development and eradication of major killer diseases. Though nationwide health plans have succeeded in reducing fatality of infectious diseases to a certain extent, however there is a significant increase in some non infectious diseases like aplastic anemia.

Aplastic anemia is a rare disorder characterized by failure of the bone marrow to produce sufficient blood cells for the circulation (NORD, 2008). The lack of blood cells produces a potentially very serious or fatal disease unless properly managed. Until about 1980 the majority of patients with severe disease did not survive more than a year but fortunately new methods of support and treatment have changed the scenario (Leukemia Research Foundation, 2005).

In last two years of our experience we have seen around 300 aplastic anemia patients for chromosomal breakage studies.

The findings of this study on Indian aplastic anemia patients along with their epidemiological and clinical evaluation have inspired us to work further. As per our experience, the disease is increasing day by day in Indian population. A large proportion of aplastic anemia patients are classified into idiopathic aplastic anemia and many of this population do not have access to laboratories which could investigate them further. Also, there is lack of specific molecular diagnostic tools, drugs and therapies to cure the disease.

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Furthermore, none of the studies have been targeted in India to rule out the causative agents/ presence of genetic lesion, if any, and development of molecular drug/s for the disease except a few studies conducted at some of the premier institutes. Even in these studies the sample size was statistically insignificant and only one particular parameter has been studied (Neelam *et al*., 2006; Ahmed, 2006; Rashmi, 2004; Gupta, 2008).

In the present study, we have tried to shed light on the health status of population of India by studying the pattern of aplastic anaemic disease, its occurrence, present status, distribution, etc across the states in India.

MATERIALS AND METHODS

The study was designed to enroll all the patients who has been catered to the hospital in duration July, 2007 to June, 2009 for classification of the inherited version of the disease viz. Fanconi's anemia to the acquired version of the disease, whereby around 300 patients of different age groups and sex was encountered to the Immunology and Molecular Biology Lab of the organization. The study was presented to and approved by the ethical committee of the Indraprastha Apollo Hospital, New Delhi and the Departmental Research Committee of the AIB, Amity University, Uttar Pradesh. An informed consent was taken for each and every patient before he /she was subjected to a common questionnaire to draw the sketch of the clinical symptoms, etiological factor /s involved in, and the pedigree of the patient. Census data were collected from ICMR, New Delhi, India and the Ministry of Health & Family Welfare, India and taken as reference to compare the population statistics for the disease.

The latest report of complete blood count and the details of whole blood and platelet transfusions of each patient were taken to classify the severity of the disease. However, out of 300 patients, 60 patients were excluded from this classification because of the unavailability of the blood count.

Sample collection: A 3-4 mL of peripheral blood sample was collected in heparinized vaccutainer (BD, Vacutainers) for each patient. An age and sex matched control of a healthy individual with no history of alcohol and smoke was taken for each patient separately.

Lymphocyte culture method: A peripheral blood culture stimulated with phytoheamagglutinin-M (Biological Industries.) for 72 h has been set-up. The clastogen Mitomycin C [MMC] (Biochem Pharmaceuticals) has been added 48 h before the termination at a concentration of 200 ng/mL of culture which was standardized in the lab. Also a peripheral blood routine culture was set-up for each patient to rule out any congenital abnormality. Age and sex matched controls samples were also set up with clastogen Mitomycin C [MMC] and peripheral blood routine culture. Termination of culture using colchicine (Biological Ind), harvesting and slide preparations were done as per the standard protocols (Sandberg, 1980).

Table 1: Statistics of epidemiology, etiology and inheritance of aplastic anaemia

Characteristic		Observation
Epidemiology		
Demography	Area wise	
	Bihar	53/191(27%)
	Delhi	46/191(24%)
	Uttar Pradesh	44/191(23%)
Age	Age wise(Years)	
	6-10	46/300(15%)
	11-15	66/300(22%)
	16-20	69/300(23%)
	21-25	34/300 (11%)
Sex	Male/Female	3.24:1
Blood count	Moderate	52/210(24.76%)
	Severe	153/210(72.86%)
	Very severe	06/210(2.38%)
Etiology		
History of Previous infection of jaundice		05/211(12%)
History of Previous infection of without jaundice		34/211(88%)
Inheritance		
Cytogenetic classification (Stress cytogenetic test)		24/261(9.20%)

Chromosome breakage analysis by MMC method: Chromosome breakage analysis was done on MMC treated slides stained with Giemsa stain, whereas the routine culture slides were processed for GTG banding. A minimum of 50 metaphases per treatment were analyzed. The MMC-induced chromosomal breakages were then compared to healthy controls. Achromatic areas less than a chromosome width i.e., gaps were excluded in the calculation of chromosomal breakage frequency. Achromatic areas more than a chromosomal width were scored as breaks. Single chromatid breaks, isochromatid breaks and acentric fragments were scored as one break each while dicentric, ring chromosomes and chromosomal breaks were scored as two breaks each. Radial configurations were scored number of chromosome breaks as number of chromosomes involved in the configuration. The proportion of breaks and radial figures was expressed in percent, i.e., number of breaks or radial figures/number of mitotic figures × 100 (Cervenka, 1981; Brown, 1997). In the present study, the comparison was done by expressing the increased breaks in patient to the control sample.

RESULTS

The result of the present study covers three different area viz. epidemiology, etiology and the inheritance of the disease (Table1).

Of 300 patients, the demographical data was available for only 220 (73.3%) patients of them a very high rate of incidences of the aplastic anemia disease have been seen from Bihar, Delhi/NCR and Uttar Pradesh,

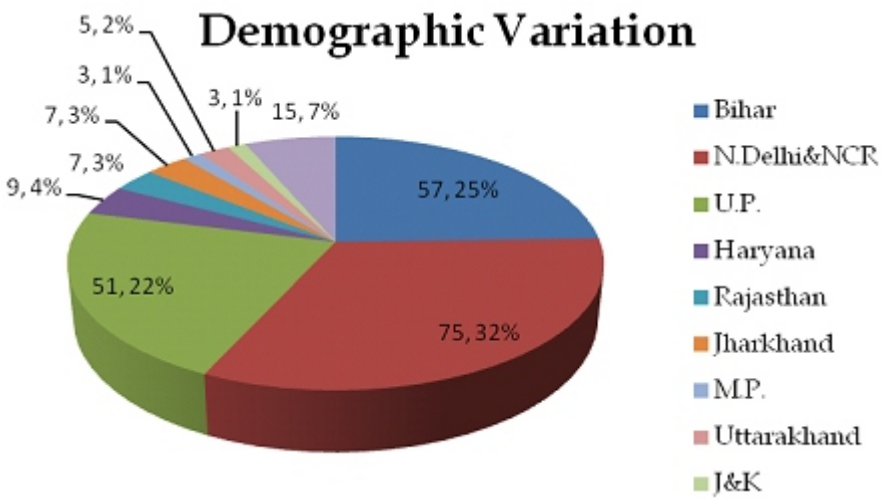

Fig. 1: Demographic distribution of aplatic anemia patient

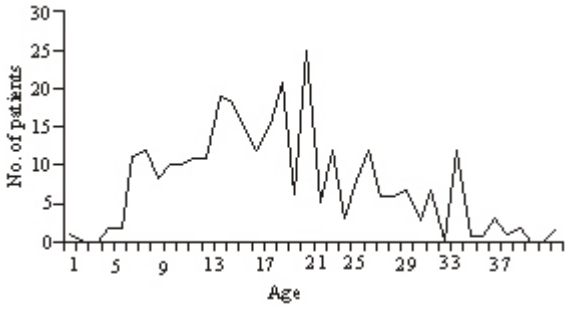

Fig. 2: Age wise distribution of aplastic anemia patient

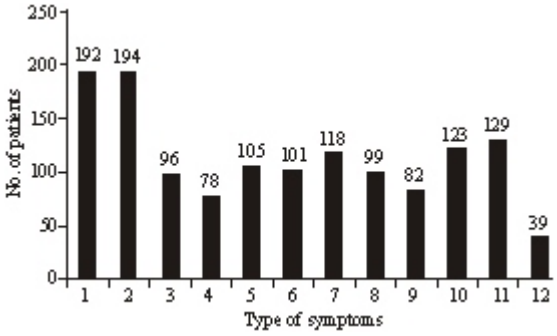

Fig. 3: Classification on the basis of the predominant symptoms

which accounts for 27, 24 and 23%, respectively, out of the 16 different states of northern India from where the study samples have been received (Fig. 1). The disease was most commonly seen in the patients that fall in the age group of 11-20 years (Fig. 2). An Imbalanced sex ratio of male to female 3.24:1 was observed in the present study. Out of 300 patients studied, the complete blood count details were available for 210 (70%) of them 24.76, 72.86 and 2.38% patients were classified as Moderate or Non-severe, Severe, and Very severe aplastic anemia on the basis of Camitta's classification. The clinical symptoms of the patient have been available and recorded for 211 patients (Fig. 3). Aplastic anemia was more common in patients from family with lower socioeconomic status.

Of 300 patients, the history of previous infection was available for only 211(70%) patients out of them 39 (13%) of the patients had evidence of infection and taken subsequent treatment for the same. Whereas around 172 patients (57%), had no history of any infection (Fig. 4).

Of 300 patients, the cytogenetic classification for chromosomal breakage study was possible only for 261 patients, of them 24 (8%) patients have shown a significant increase in number of breaks in comparison to their control. The Fig. 5 to 8 shows the chromosomal structural anomalies studied in the present study.

DISCUSSION

The Apalstic anemia is being considered as a rare disease with very low incidences in France to a very high incidence rates in countries like Sweden. The exact incidences of disease in published literature in India are still unknown. Study conducted by Ahmed *et al.* (2006), showed disease incidence 6.8% in Lucknow, India but this had limitations of age groups and the numbers of patient's included.

The present study was conducted on 300 patients, which have been referred from premier institute of Delhi state and its periphery. Even in present study the exact incidence rate of the disease cannot be calculated but the amount of disease samples received in the laboratory in last consecutive three years (2007- 2009) was 26, 29,

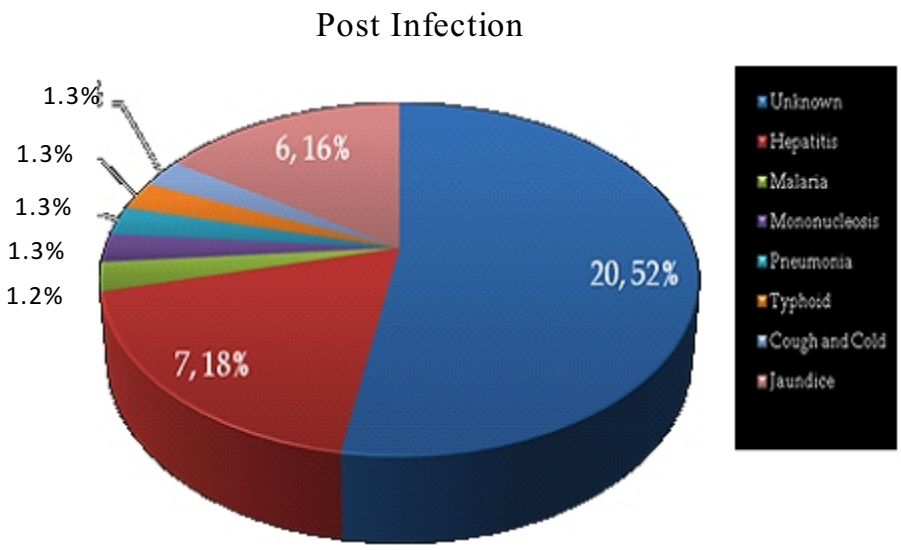

Fig. 4: Previous history of infection

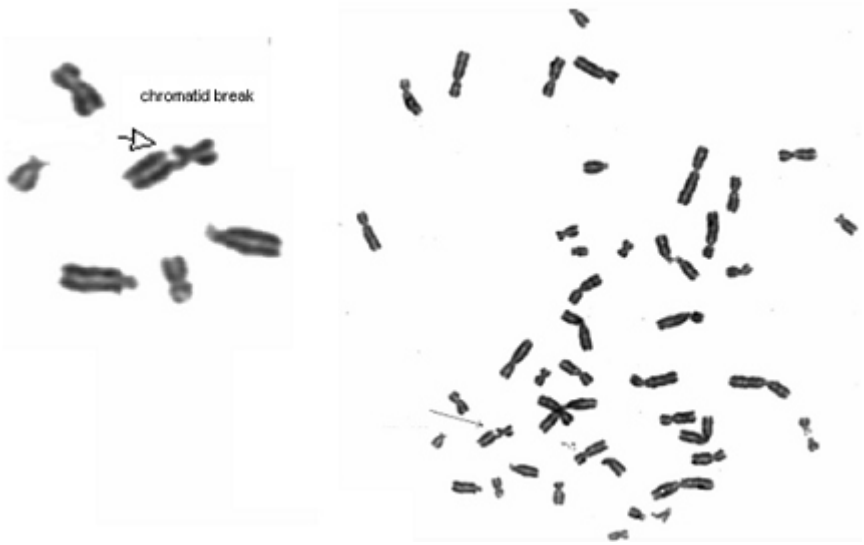

Fig. 5: Metaphase showing chromatid break

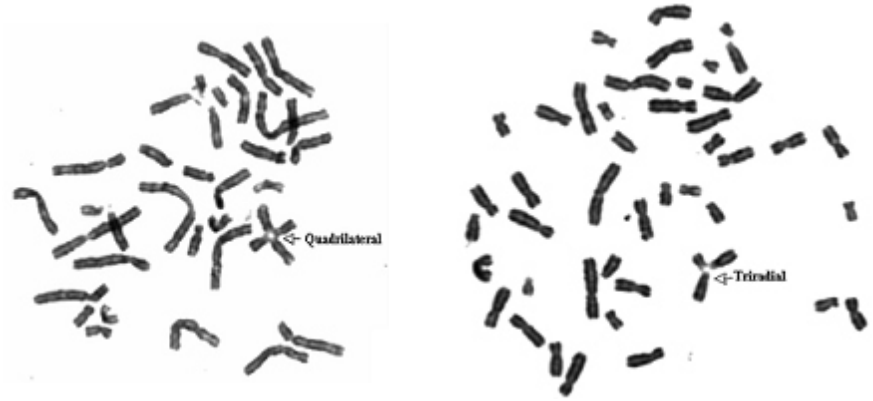

Fig. 6: Metaphase showing triradial and quadrilateral

Fig. 7: Metaphase showing complex figures

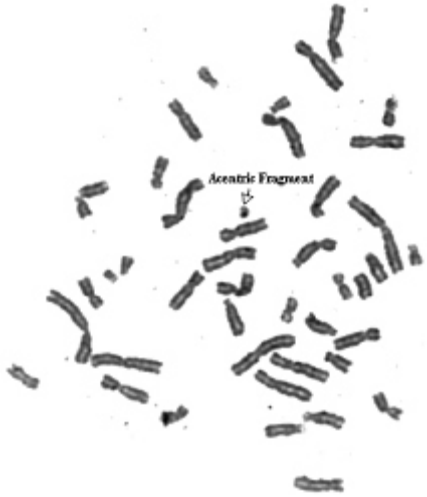

Fig. 8: Metaphase showing acentric fragment

44%, respectively, which showed a steep rise in the incidences of the study. To the best of our knowledge the prevalence of aplastic anemia has not been calculated in India, so far.

According to the literature the male to female ratio of the affected individual is 1:1. However, the study conducted by Gordon (1989) and in India by Ahmed *et al.* (2006) showed a higher ratio of male to female and correlated it with a higher risk of environmental exposure to potential toxic agents in males than females. In the present study the disease was found to be 3.24 times higher in male than female. The probable region could be the male dominant social culture of the country. The complete blood count reports of around 70% patients were suggested severe condition of the disease. The disease is more common in northern states Viz. Bihar, Uttar Pradesh and Delhi. The disease was common in the age group of 11-20 years old patients whereas the study conducted by Neelam *et al.* (2006) showed a median age of 8 yrs for 54 cases out of 94 cases of Aplastic Anemia.

The etiological factors for acquired aplastic anemia have been reported in many forms like pesticides, drug, and toxins and radiation. In India the study conducted on 25 patients showed lack of association between disease and organochlorines exposures (Ahmed *et al.*, 2006). In the present study, the data showed around 39 patients had evidence of infection of them 5 patients had evidence of viral hepatitis which almost excludes the viral infection from the etiology of the disease progression. Hence, it alarms the need to find out the molecular lesion present in these patients.

The inherited disease like fanconi's Anemia have been seen in the present study was 9.20% of the patients from all age groups which has a slight difference from other studies conducted in the country. A comparatively large study on 94 aplastic anaemic patients of all age groups by Neelam *et al.* (2006), showed 13.8% patients with Fanconi's anemia whereas another study by, Gupta *et al.* (2008), suggest 11.3%. The differences in the population affected by inherited diseases like fanconi's anemia either may be because of statistically low population included in the previous studies, or the steep increase in the idiopathic acquired aplastic anemia which might alter the ratio of inherited to acquired disease.

The present study would help to understand the increased incidences of aplastic anemia in the country and suggests the need of molecular studies of the patients which can subsequently used to design molecular drugs for the disease.

CONCLUSION

In the present study, we have tried to shed light on the health status of population of India by studying the pattern of aplastic anaemic disease, its occurrence, present status, distribution, etc across the states in India. The outcome of the present study may or may not be helpful in defining the etiology and the treatment strategies for the disease but study of disease incidences, will provide us data which will help in formulation of strategies for prevention or protection and also may provide important insights into genes important in hematopoiesis and help to identify therapies applicable to both acquired and inherited conditions.

ACKNOWLEDGMENT

We thank the individuals with Aplastic anemia, their families and their physicians for their cooperation. We

acknowledge the support from Department of Hematology, All India Institute of Medical Sciences, New Delhi for referring aplastic anemia patient to our centre. The help from all our laboratory members and trainees is appreciated in preparing figures and data compilation.

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