

## Serum Homocysteine Level in Children with Juvenile Idiopathic Arthritis

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### Abstract

**Background:** Juvenile Idiopathic Arthritis (JIA) is the most common chronic rheumatic disease of childhood and a leading cause of short and long-term disability. Abnormal level of serum homocysteine (Hcy) may be found in Juvenile idiopathic arthritis (JIA) patients. Some studies were done on serum homocysteine levels in children with JIA which showed conflicting results. So far, no study regarding the Hcy level in JIA patients was done in Bangladesh.

**Objectives:** The study was aimed to assess the homocysteine level in children with different subtypes of JIA.

**Methods:** It was a cross sectional and case control study. Fifty newly diagnosed cases of JIA attending the Pediatric Rheumatology Clinic of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from January 2016 to March 2017 were included in the study. Fifty age and sex matched controls were selected. Serum homocysteine levels were measured in both cases and controls.

**Results:** Among JIA patients, 36% had high serum homocysteine (Hcy) level whereas 100% of controls had normal level. Mean hcy level was significantly ( $p < 0.05$ ) higher among cases than controls. Significantly higher number and level of hyperhomocysteinemia was found among JIA patients having duration of illness more than 1 year than duration less than 1 year. Mean homocysteine level of polyarticular RF positive cases was found as  $14.38 \pm 3.79$   $\mu\text{mol/L}$  and that of SJIA was found as  $13.0 \pm 4.66$   $\mu\text{mol/L}$ . When compared with homocysteine levels of control group these two groups had significantly higher homocysteine level.

**Conclusion:** More than one third of JIA patients had hyperhomocysteinemia. Mean hcy level of JIA cases was significantly higher than that of controls. Significant association of hyperhomocysteinemia was present with duration of illness and certain types of JIA.

**Keywords:** Juvenile Idiopathic Arthritis, Serum homocysteine level, Polyarticular, Hyperhomocysteinemia

### Introduction

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease of children. JIA is characterised by idiopathic peripheral arthritis with an immunoinflammatory pathogenesis possibly activated by contact with external antigens. Worldwide incidence of JIA ranges from 0.8 to 22.6 per 100,000 children per year with the prevalence ranges from 7 to 401 per 100,000 children. JIA is classified into seven subgroups according to International League of Association for Rheumatology (ILAR) 2001 classification.<sup>1</sup>

Homocysteine (Hcy) is a sulphhydryl amino acid derived from the essential amino acid methionine during its conversion to cysteine.<sup>2</sup> Main metabolic pathway of Hcy requires folic acid, vitamins B6 and B12. Its concentration depends on age, gender and ethnic background.<sup>3</sup> Homocysteine can impair bone health by interfering with osteoclastic activity. Furthermore, homocysteine induces

apoptosis of human bone marrow stromal cells, thereby impairing bone repair.<sup>4,5</sup> It is reported that an increased homocysteine level is a strong and independent risk factor for osteoporotic fractures in older people.<sup>5</sup>

Different genetic and acquired factors may lead to an elevated homocysteine level.<sup>6</sup> Acquired factors are more common than genetic disorders, and include vitamin B12, B6 and folate deficiency and side effects of some drugs (methotrexate, phenytoin, carbamazepine and many more).<sup>7</sup> As JIA is a chronic inflammatory disease, there could be some sort of multivitamin deficiencies specially vitamin B12, B6 and folate. There are some studies done on the level of Hcy in children with JIA but the results have been conflicting.<sup>8,9</sup> No study is available in Bangladesh concerning JIA and homocysteine level. The aim of this study was to estimate the serum homocysteine level in different subtypes of JIA patients. Specific objectives were to compare the homocysteine level in different subtypes of JIA with controls, also assessed relationship between duration of disease at presentation with homocysteine level.

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## Materials and Methods

In this case control study, newly diagnosed fifty cases of JIA according to International League of Associations for Rheumatology (ILAR) attending paediatric rheumatology follow up clinic and paediatric ward, BSMMU during January 2016 to March 2017 were included. Purposive sampling was done to meet the inclusion criteria. Patients were excluded if they had either an additional chronic disease (endocrine, neurological, cardiac, renal or liver disease) that could affect homocysteine metabolism, or were receiving treatment including methotrexate or vitamins like B6, B12 and folic acid. Fifty age and sex matched controls were selected from the out patient department who attended the hospital because of acute illness other than JIA or bone disease, and who were not taking supplements of any vitamins or folic acid. Ethical approval was obtained from the Institutional Review Board of BSMMU. Informed written consent was obtained from all the participants or parents. Information were obtained from their medical records, which included age, sex, duration of disease and disease sub-type according to the ILAR classification criteria.

With all aseptic precaution 2 ml venous blood samples were drawn for measuring homocysteine levels for both the cases and controls. Serum was separated by centrifugation within 30-45 mins of collection and was preserved in ultra-freezer at -20 degree centigrade after labeling. Serum homocysteine level was determined by chemiluminescence microparticle immune assay (CMIA) using the analyzer The Architect system Homocysteine assay, Abbot; Germany 2008 in the Department of Biochemistry, BSMMU. There are two studies about the level of Hcy in children with JIA and the results have been conflicting.<sup>8</sup> Normal serum level of homocysteine in adults is usually 5-15 mol/l, with a mean level of 10µmol/l according to the standard methods.<sup>10</sup> The levels of hcy between different types of JIA and different disease duration were compared in the present study. Hcy levels between cases and controls were also compared.

All statistical analyses were done using the statistical software SPSS 23.0. The Chi-square test, student t-test and scatter diagram was applied to evaluate the association between the variables when indicated. A *p*-value of less than 0.05 was considered as significant at a 95% confidence interval.

## Results

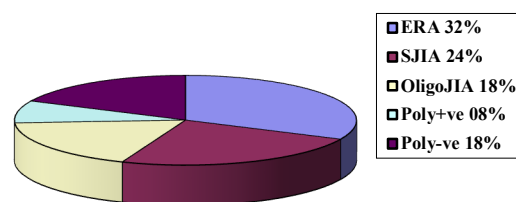
It was observed that majority (44.0%) patients belonged to the age group of 11-15 years among the cases with a mean age 8.96±4.25 years (Age

range: 1.0 year to 16 years). Majority (58.0%) patients were male among cases (table I). The mean age of controls was 9.72 years (Age range: 1.2 to 15 years) and among them 29 (58.0%) were male and 21 (42.0%) were female.

**Table I:** Study population of the demographic variables (n=50)

Demographic variables	Case group (n=50)	
	n	%
Age (years)		
≤5	13	26.0
6-10	13	26.0
11-15	22	44.0
≥15	2	4.0
Mean±SD	8.96	±4.25
Range (min-max)	1.0	-16.0
Sex		
Male	29	58.0
Female	21	42.0
Male: female	1.38:1	

Five subtypes of JIA patients were found (figure 1) including ERA (32.0%), systemic JIA (24.0%), oligo-articular JIA (18.0%), poly-articular RF negative (18.0%) and poly-articular RF positive JIA (8.0%). In the JIA group (cases), homocysteine level was normal in 64.0% and high in 36.0%. Among control group, all had normal homocysteine levels (table II).



**Figure 1:** Sub-types of JIA among the cases.

**Table II:** Homocysteine level among case and control

Level of homocysteine	Case (n=50)		Control (n=50)		<i>p</i> value
	N	%	n	%	
5-15 µmol/L	32	64.0	50	100.0	
>15 µmol/L	18	36.0	0	0.0	0.024 <sup>s</sup>
Mean±SD	11.43	±4.29	9.08	±2.28	
Range (min-max)	6.53	-20.52	5.33	-12.5	

s= significant

The mean homocysteine level of cases was significantly higher than controls. High level of homocysteine was found in more than two-thirds (69.2%) of patients having duration of illness more than 1 year. This was statistically significant (table III).

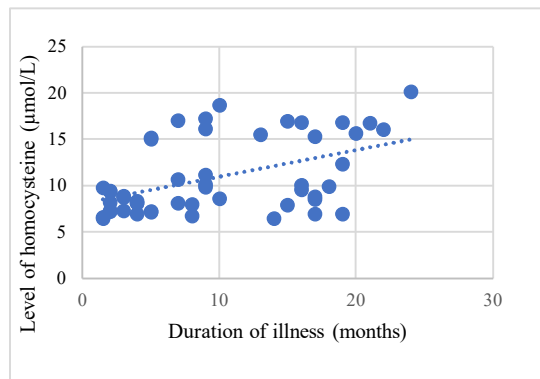
**Table III:** Association between homocysteine level with duration of illness at diagnosis (n=50)

Duration of illness	Level of homocysteine				p value
	5-15 $\mu\text{mol/L}$ (n=32)		>15 $\mu\text{mol/L}$ (n=18)		
	N	%	n	%	
<6 month (n=15)	13	86.7	2	13.3	
6 month-1 year (n=22)	15	68.2	7	31.8	
>1 year (n=13)	4	30.8	9	69.2	
Mean $\pm$ SD	9.31 $\pm$ 5.61		13.36 $\pm$ 7.51		0.035 <sup>s</sup>
Range (min-max)	1.5-24		2-24		

s= significant

p value reached from chi square test

The scatter diagram showed that homocysteine levels increased significantly with the increased duration of disease at onset (figure 1). Spearman correlation coefficient was: 0.476 which was statistically significant ( $p=0.001$ ).

**Figure 2:** Between duration of illness at presentation and Homocysteine Levels.

When compared with homocysteine level of controls, polyarticular RF positive JIA and systemic JIA patients had significantly higher homocysteine level (table IV)

**Table IV:** Comparison of Homocysteine Levels of Different Subtypes of JIA with Controls (n=50+50)

Type of JIA	Level of homocysteine				p value
	Cases		Controls		
	N	Mean $\pm$ SD	n	Mean $\pm$ SD	
Oligoarticular	9	11.65 $\pm$ 4.55	50	9.08 $\pm$ 2.28	0.051 <sup>ns</sup>
Polyarticular RF positive	4	14.38 $\pm$ 3.79	50	9.08 $\pm$ 2.28	0.001 <sup>s</sup>
Polyarticular RF negative	9	11.54 $\pm$ 3.99	50	9.08 $\pm$ 2.28	0.096 <sup>ns</sup>
SJIA	12	13.0 $\pm$ 4.66	50	9.08 $\pm$ 2.28	0.003 <sup>s</sup>
ERA	16	8.45 $\pm$ 2.38	50	9.08 $\pm$ 2.28	0.425 <sup>ns</sup>

s= significant, ns= not significant

p value reached from unpaired t-test

## Discussion

Homocysteine (Hcy) is a sulphhydryl amino acid derived from the essential amino acid methionine during its conversion to cysteine, which is a marker of severity of arthritis in JIA patients.<sup>2</sup> Its concentration in the plasma or serum is about 10  $\mu\text{mol/L}$ . A plasma homocysteine concentration exceeding 15  $\mu\text{mol/L}$  is termed as hyperhomocysteinaemia in adults.<sup>10,11</sup> Not much data exist on Hcy levels in children.<sup>10</sup> One study found that Hcy levels in 8-12 year old boys and girls were about half of that found in adults.<sup>12</sup> There was no sex difference and the Hcy distribution was nearly Gaussian, with a mean level of 5.25  $\mu\text{mol}$  and the reference range was from 2.9 to 7.6 $\mu\text{mol}$ . Another study showed that distribution of Hcy levels in children was substantially lower than that of adults.<sup>3</sup>

Few studies done on the level of homocysteine (Hcy) in children with JIA found conflicting results.<sup>8,9</sup> But it is believed that there is a relationship between blood homocysteine levels and severity, duration and subtypes of JIA.<sup>8</sup> Present study assessed the Hcy level of JIA patients and compared with controls in a tertiary care hospital in Bangladesh. Though it is assumed that the Hcy level in children is lower than that of adults; as no standard is available, this study considered adult standard to define hyperhomo-cysteinaemia.

Most of the cases (44.0%) were within 11 to 15 years age group which supports the juvenile onset of the disease. Among the cases, male: female ratio was 1.38:1. A prospective observational study carried out in the same set up also found more males than females (M:F ratio approximately 2:1), and most of the patients were in the age group of 11-15 years.<sup>13</sup>

This different result regarding sex distribution and types of JIA found in this study could be due to social or geographical difference. In the present study, maximum number of patients were suffering from ERA (32.0%), followed by SJIA, polyarticular and oligo articular JIA (figure 1). This order of frequency probably reflects the higher attention to male children in our perspective. ERA was found as the predominant type in some other studies done in India and Taiwan as well.<sup>14,15</sup>

The present study is consistent with the study of Huemer et al where JIA patients had significantly higher level of Hcy than that of controls.<sup>8</sup> Goncalves et al compared the plasma Hcy concentration between 51 JIA patients with 52

healthy controls. The mean plasma Hcy concentration was higher in JIA patients than in healthy controls ( $p = 0.615$ ). Significantly higher concentration of Hcy was observed in the subgroup of 13 – 18 years ( $p < 0.001$ ).<sup>16</sup> As because, our study did not subgroup the JIA patients according to age, this comparison could not be done.

Association of homocysteine level with duration of illness was seen in the present study and it was found that 69% of JIA patients with disease duration more than one year at presentation, had hyperhomocysteinemia. No documented reports were available for comparison of this finding. But if the mechanism of hyperhomocysteinemia in arthritis is analyzed; it is found that there is reduction in circulating levels of pyridoxal 5'-phosphate (PLP), the biologically active form of vitamin B<sub>6</sub> in rheumatoid arthritis. This low level of PLP ultimately raises the Hcy level.<sup>17</sup> So, it can be extrapolated that, as duration of illness increases PLP level continues to decrease further which in turn increases the Hcy level.

Although the mean Hcy level was within the normal range, polyarticular seropositive JIA and systemic JIA patient's Hcy levels were significantly higher than that of the controls. Perhaps it was due to the natural history of the serotypes having worst disease course and potential morbidity. Our findings were consistent with Pietriwicz et al study who found significantly higher homocysteine level in JIA patients compared to control group and even higher homocysteine level in polyarthritis group compared to oligo arthritis patients.<sup>18</sup>

Pathogenesis of juvenile idiopathic arthritis (JIA) includes increased inflammatory markers, homocysteine, lipids levels and inflammatory cytokines.<sup>19</sup> It is hypothesized from a post mortem study that an excess of homocysteine may play a significant role in the development of atherosclerotic lesions.<sup>20</sup>

At present, there are many known risk factors for developing atherosclerosis. Some of them are amenable and may be modified, while some are non-amenable and cannot be modified; however, it is possible to identify groups at increased risk for cardiovascular diseases. Considering the fact that clinical consequence of the atherosclerotic process begins early in childhood but manifest during adult life, the introduction of appropriate preventive measures in children belonging to the risk group for cardiovascular disease and osteoporosis, is important. These measures may

halt the development of the atherosclerotic process and other complications including osteoporosis at an early stage.

## Conclusion

It is evident that a considerable number of JIA patients had hyperhomocysteinemia considering the adult normal mean level as standard. Further study with larger sample size on new-onset JIA patients are recommended to explore the association between serum homocysteine levels and the disease.

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