ORIGINAL ARTICLE

METABOLISM OF NITRIC OXIDE AND LIPID PEROXIDATION IN CHIDREN WITH PERTHES` DISEASE AND TRANSIENT SYNOVITIS OF THE HIP JOINT

Tauseef Raza[1], Sergei Borisivich Nazarov[2], Irina Konstatinovna Tomilova[3], Aleksei Valerivich Philosophov[4]

[1]Department of Orthopaedics, KMU Institute of Medical Sciences, Kohat - Pakistan

[2]Department of Physiology, Ivanovo State Medical Academy, Russia

[3]Department of Biochemostry Laboratory, Scientific Research Center, Ivanovo State Medical Academy, Russia

[4]Department of Orthopaedic Children Clinical Hospital No 1, Ivanovo, Russia

ABSTRACT

Objective: To study parameters of metabolism of nitric oxide (NO) and lipid peroxidation - malondialdehyde (MDA) and conjugated dienes (CD) in children with Perthes disease (PD) and Transient synovitis of the hip joint.

Material and Methods: This study was conducted at Children Clinical Hospital No 1, Ivanovo, Russia from November 2012 to February 2014. A total of one hundred and thirty six children (sixteen children from control group), and one hundred and twenty one children diagnosed as PD and TS were examined. In the whole blood and plasma nitrate ions (NI) were analyzed by electrichemical method. Parameters of lipid peroxidation were measured in plasma, malondialdehyde (MDA) by tiobarbiturate method, and conjugated dienes (CD) by spectrophotometric method. Patients data was processed statistically using SPSS version 14.

Results: In TS data analysis revealed reliable increase in the concentration of NI in the whole blood (1,5 ± 0,15) and (1,6 ± 0,16) in the plasma, as compared with the control group and patients with stage I of PD. In PD the products of NO changed depending upon the stage of disease. In stage I the concentration of NI constituted (1,90 ± 0,09) in the whole blood, and (1,8 ± 0,11) in the plasma. In TS the parameters of lipid peroxidation: MDA constituted 7,5 ± 0,38 in the plasma. In Perthes disease MDA and DC changed with the disease progression. There was reliable increase in MDA, comparing both with control group and TS during stage I of disease and constituted (8,6 ± 0,44). There was a reliable increase ($p < 0,05$) in the parameters of NI and lipid peroxidation (MDA), both in TS and PD as compared with the control group, but also in between the TS and stage I of PD.

Conclusion: In children with PD parameters of end products of NO and lipid peroxidation (MDA) were twice raised in comparison with the control group, and authentically differed from patients with TS during stage I of the disease.

Key Words: Perthe's` disease, transient synovitis, nitric oxide, malondialdehyde, conjugated dienes.

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INTRODUCTION

Early diagnosis and treatment of Perthes disease (PD) is still a problem and remains controversial[1-5]. In the early stage of PD, the differential diagnosis is usually carried out with Transient synovitis of the hip joint (TS)[6-8]. Synovitis is an obligatory condition, present in the pathogenesis of both diseases[9-11]. It is not possible to make prognosis regarding dystrophic changes in the head of femur depending only upon clinical and radiological findings[12-17].

No laboratory tests are currently available for early diagnosis of PD. Blood tests are usually normal in PD. One of the markers of aseptic inflammation is nitric oxide (NO)[18,19]. Role of NO has been proven in key inflammatory mechanisms[20-22]. Zidek et al[23] in there experimental study showed that change in concentration of NO is one of the paramount parameter of inflammation in every

Dr. Tauseef Raza (Corresponding Author)
Assistant Professor & In charge
Department of Orthopaedics, KMU Institute of Medical Sciences, Kohat - Pakistan
Cell: +92-311-6777765
Email: drtauseefraza@gamil.com
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disease process. No studies are available regarding metabolism of NO in PD and TS.

Wide range of physiological effects of NO is realized through various mechanisms, and in particular through participation in processes of lipid peroxidation[24,25]. It is considered, that one of the parameters of inflammation during dystrophic diseases in adults is the change in content of malonic dialdehyde (MDA) - end metabolite of nonenzymic degradation in lipid peroxidation[26]. For lipid peroxidation we measured products such as malondialdehyde (MDA), and conjugated dienes (CD). We studied the interaction of metabolism of NO and products of lipid peroxidation for better understanding of the mechanisms involved in progression and chronization of processes in Perthes disease. Determination of these parameters in PD and TS would help in timely diagnosis, treatment and prophylaxis of complications.

MATERIAL AND METHODS

This study was conducted in the department of traumatology and orthopedics, children clinical hospital No 1, Ivanovo, Russia from November 2012 to February 2014. Total of 136 children of both genders including 71 children with Perthes disease, 49 children with Transient Synovitis, and 16 healthy children of the control group were included in the study. Age of patients with PD ranged from 5 to 14 years (mean age: 6.5 years), 3 to 10 years in TS (mean age 5.8 years), and 5 to 12 years in the control group (mean age: 6.2 years). Children suffering from any other acute or chronic diseases were excluded from the study. Blood sampling was done from the ulnar vein in strict aseptic conditions. Blood was taken early in the morning before breakfast. Convenient sampling methods were used to collect the data. Laboratory tests were done in the scientific research center of Ivanovo State Medical Academy, Russia. Informed consent was taken and the study was duly approved by the ethical committee of hospital. Data was collected on a specially designed proforma.

Nitric oxide was studied by measuring the stable end product of NO metabolism – nitrate ions. They were measured by electrochemical method of detection using the ion selective electrodes[27]. Parameters of lipid peroxidation- conjugated dienes (CD) were measured by spectrophotometry, and malondialdehyde (MDA) by thiobarbituric method. All the findings were documented on proforma and were subjected to statistical analysis by using software SPSS version 14 and p value of < 0.05 was considered significant.

RESULTS

By analyzing data of 136 (71 patients with PD, 49 with TS, and 15 in the CG). Table 1 is showing the reliable increase in concentration of nitrate ions in children with TS and PD ($p < 0,05$), in comparison with the CG. In children with PD there was a tendency showing decrease in nitrate ions from stage I to stage IV ($p < 0,01$). Maximum increase was observed in stage I of PD ($1,9 \pm 0,09$; $p < 0,01$ w/b), and ($1,8 \pm 0,11$; $p < 0,01$ in pl). In stage IV the concentration of nitrate ions was decreased to $1,11 \pm 0,10$ in the whole blood; and $1,23 \pm 0,18$ in the plasma ($p > 0,05$). Reliable increase in stage I in the concentration of nitrate ions was observed not only in comparison with the CG but also with TS ($p > 0,05$). The parameters of lipid peroxidation (MDA and CD) are presented in Table 2.

Both parameters of lipid peroxidation (MDA and CD) were significantly increased in patients with TS, in comparison with the control group and constituted $7,5 \pm 0,38$ and $2,9 \pm 0,27$ ($p > 0,05$) respectively. The

Table 1: Nitrate ions in whole blood (w/b) and plasma (pl) in PD, TS and CG

Parameter	No. of patients	nitrates (w/b)	nitrates (pl)
CG	16	0,6 ± 0,04	0,7 ± 0,03
TS	49	1,5 ± 0,15*	1,6 ± 0,16*
PD I	12	1,9 ± 0,09*	1,8 ± 0,11*
PD II	27	1,7 ± 0,15*	1,6 ± 0,13*
PD III	21	1,4 ± 0,14*	1,5 ± 0,18*
PD IV	11	1,1 ± 0,10**	1,2 ± 0,18**

Table 2: Parameters of lipid peroxidation (MDA,CD) in patients with PD and TS

Parameter	No. of patients	MDA nmole/ml	CD IU/mg
CG	16	4,6 ± 0,22	1,8 ± 0,17
TS	49	7,5 ± 0,38*	2,9 ± 0,27*
PD I	12	8,6 ± 0,44	3,4 ± 0,19*
PD II	27	6,7 ± 0,52*	3,5 ± 0,53*
PD III	21	5,8 ± 0,31*	1,7 ± 0,15
PD IV	11	5,3 ± 0,45	1,6 ± 0,39

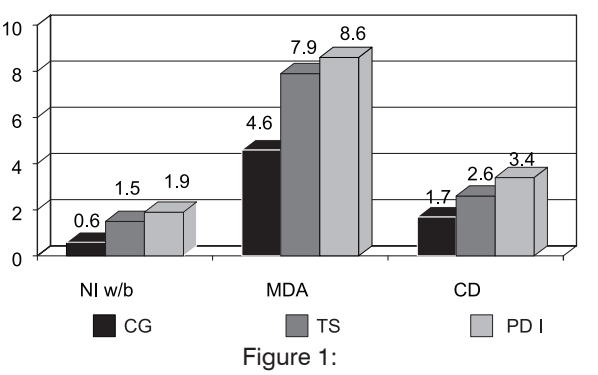

Figure 1:

same parameters in PD changed depending upon the stage of the disease. Analysis of the data revealed that the parameters of lipid peroxidation varied according to the severity of dystrophic changes in the afected joint. In stage I of PD when the dystrophic changes are considerably expressive, the concentration of MDA and CD were also signifintly higher as compared with the CG (MDA - 8,6 ± 0,52; CD - 3,5 ±0,63; $p > 0,05$). From stage I to IV of PD, there was tendency showing stabilization of the parameters as the patient improved clinically (5,3 ± 0,45 MDA; 1,6 ± 0,39 CD $p > 0,05$). MDA was significantly increased in stage I, not only in comparison with the CG but also with TS ($p > 0,05$). CD on the other hand didn't showed reliable difference in stage I in comparison with TS. The graphic expression showing the relationship between NI in the whole blood, and MDA, CD in the plasma is shown in Figure 1.

DISCUSSION

The analysis of results shows that in children with Perthes' disease the parameters of end product of nitric oxide metabolism (NI), and parameter of lipid peroxidation (MDA) were not only reliably increased as compared with the control group, but also in comparison with the patients of TS. This may serve as a diagnostic marker in the differenrial diagnosis of PD and TS.

These results confirms the findings of Klebanov et al.30 that enhanced functional activity of cell results in increased production of various bioactive compound (nitric oxide, superoxide anion - radical, hypochlorite - ion, etc). Some of them cause bactericidal effect[31], and are also capable to influence microcirculation[32]. Nitric oxide is the precursor of so-called endothelium derived relaxing factor (EDRF) Kozlov et al[32] which causes vasodilation and improvement in microcirculation due to rexation of smooth muscles.

Production of NO can considerably increase in various diseases[33,34]. Inducible NO-synthase is responsible for synthesis of NO in leukocytes[31,35]. Production of inducible NO-synthase can occur in the phagocytes in pathological tissues, for example in focal inflammation. Besides, this enzyme is constantly present in small amounts in phagocytes, and upon activation increases the activity of cell enzymes, alongside with the synthesis of NO-synthase[31]. Hence, certain contribution to changes in microcirculation can bring, in particular, nitric oxide, secreted by leukocytes in the circulation.

Wide spectrum of physiological effects of NO take place through different mechanisms and partially through participation in lipid peroxidation[36-38]. Excessive production of NO may initiate the action of this mediator through free radical mechanism, causing activation of processes of lipid peroxidation[39]. High concentration of nitric oxide causes vasodilation[40-42], increased vascular permeability, leading to edema and subsequent development of inflammatory reaction in PD and TS. In generation of vasogenic edema lies the reaction between NO and O2, leading to formation of potential toxic peroxide nitrite (ONOO-)[30,43,44] which induces necrosis of tissues. Hence, hyperproduction of nitric oxide is one of key components in oxidative stress.

It is well known, that increase in concentration of nitric oxide is a compensatory mechanism, improving blood circulations in pathological conditions[45]. However, excessive production of NO stimulates apoptosis, due to toxic influence of surplus NO on cells[46-48], bringing in deterioration of microcirculation. This in case of TS and PD excessive production of NO can manifest as increased joint effusion, resulting in hypertension and slowing down of venous outflow from the hip joint.

The changes in the parameters of end products of NO, and lipid peroxidation in patients with PD shows decrease in the antioxidant defense mechanisms, depending upon expressiveness of dystrophic processes, and presence of productive inflammation in the hip joint. The increase in parameters of metabolism NO and lipid peroxidation specifies increase in pro oxidant effects of nitric oxide during progression of disease and depends upon the stage and clinical course of pathological process.

Thus, the analysis of data confirms presence of close pathogenic connection between increased formation of NO and processes of lipid peroxidation, as well as clinical-laboratory manifestation of TS and PD, both on systemic and local levels. Significant role of NO in pathological and physiological processes, in combination with parameters of lipid peroxidation provide the unique diagnostic opportunities for timely diagnosis and treatment of Perthes disease.

CONCLUSION

Parameters of metabolism of NO and lipid peroxidation (MDA, DC) in chidren with TS and PD can be used as a diagnostic method and for monitoring the course of treatment. In children with PD parameters of end products of NO and lipid peroxidation (MDA) were twice raised in comparison with the control group, and authentically differed from patients with TS during stage I.

RECOMMENDATIONS

Parameters of metabolism of NO and lipid peroxidation (MDA, DC) in chidren with TS and PD can be used as a diagnostic method, and for monitoring the course of treatment.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

Raza T: Concept and design, acquisition of data.

Nazarov SB: Drafting of manuscript.

Pahrova OA: Data collection and analysis.

Philosophov AV: Overall supervision and bibliography.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.