CONTENTS

ORIGINAL ARTICLE

PERIODONTAL DISEASE AND SALIVARY OXIDATION STRESS IN CHILDREN WITH LYMPHOGRANULOMATOSIS

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ABSTRACT

The aim: To investigate the impact of lymphogranulomatosis (LGM) and periodontal disease on salivary lipid peroxidation and enzymatic antioxidants` levels in children.

Materials and methods: 45 children aged 6–16 years with LGM were examined before hematologic therapy (group LGM 1), after therapy (group LGM 2), and at the remission (group LGM 3). The control group included 70 healthy children. Periodontal state of children, saliva thiobarbituric acid reacting substances (TBARS), superoxide dismutase (SOD) and catalase were examined.

Results: 6-11 years old children from LGM 1 group showed a higher frequency of periodontal disease (50,0%), as well as 12-15 year olds (80,8%) compared to healthy children (17,4% and 42,8% accordingly, $p<0,05$). TBARS levels were higher in LGM 1-3 groups of children with periodontal disease (9,79, 12,3 and 12,6 umol/l, $p<0,01$) compared to counterparts without it (8,01, 10,1 and 11,6 umol/l, $p<0,01$) and healthy children with periodontal disease (7,9 umol/l, $p<0,01$). SOD activity was higher in LGM 1-3 groups of children with periodontal disease (-0,075, -0,086, -0,074 units) compared to children without it (-0,048, -0,059, -0,04 units, $p<0,01$) and healthy children with periodontal disease (-0,04 units, $p<0,01$). Catalase activity was lower in LGM 1-3 groups of children with periodontal disease (6,72, 5,2 and 6,7 units) compared to counterparts without it (7,3, 3,7 and 4,7 units, $p<0,01$) and healthy children with periodontal disease (7,1 units, $p<0,01$).

Conclusions: Children with periodontal disease related to LGM had higher TBARS levels, SOD activity and lower catalase activity in saliva. Both LGM and periodontal disease altered lipid peroxidation and antioxidant protection in saliva of children.

KEY WORDS: children, periodontal disease, oxidative stress, antioxidant, lymphogranulomatosis

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INTRODUCTION

Lympogranulomatosis (LGM) or Hodgkin`s lymphoma is considered as a highly curable blood malignant disease with distinct clinical, histological, and biologic manifestations [1,2]. One of the pathogenetic mechanisms of cancerogenesis involves high levels of reactive oxygen species because of metabolic and signaling abnormalities [3]. Oxidation stress develops as a result of imbalance between lipoperoxidation and antioxidant system. Superoxide anion radicals cause lipoperoxidation in the plasma membrane of surrounding host cells leading to the production of hydroxynonenal and malondialdehyde, the basic components of thiobarbituric acid reacting substances (TBARS). The antioxidant system contains both enzymatic and non-enzymatic antioxidants, and the main enzymes are superoxide dismutase (SOD) and catalase.

It has been shown that the patients with non-Hodgkin`s lymphoma at the permission phase had a higher level of reactive oxygen metabolites and a lower antioxidant potential in serum as compared to healthy volunteers [4].

Children with blood malignancies have different oral manifestations, but only the study of Simon et al. [5] dealt with this periodontal disease related to LGM. Moreover, many studies have been conducted to identify the correlation between oxidative stress and periodontal disease, in which the markers of oxidative stress are examined in saliva, blood and gingivae. The non-invasive sampling makes saliva particularly useful in the research on children, especially with oral manifestations of malignant diseases. Hendi et al. [6] showed that the periodontal disease-active patients had higher catalase and lower superoxide dismutase activity as compared to the periodontal disease-free counterparts. The total antioxidant capacity level in saliva increased in children aged 3-18 years with higher gingival bleeding [7]. However, Ghallab et al. [8] demonstrated superoxide dismutase decrease in gingival crevicular fluid of aggressive and chronic periodontitis patients. In addition,

Tóthová et al. [9] claimed that salivary TBARS related to papillary bleeding index in children.

The authors hypothesized that both LGM and periodontal disease have the impact on salivary lipid peroxidation and enzymatic antioxidants` levels in children.

THE AIM

To investigate the impact of LGM and periodontal disease on salivary lipid peroxidation and enzymatic antioxidants` levels in children.

MATERIALS AND METHODS

45 children aged 6–16 years who had been diagnosed with LGM and underwent hematological treatment in the Pediatric Hematology Ward of Children Hospital (Poltava, Ukraine) were included in this study. This cross-sectional study was carried out in 2013-2019 in Poltava, Ukraine in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Bioethical Committee of PDMU, and parents received a complete explanation about the aims and methods of the study, and signed written consent. Patients were treated according to international protocols of chemotherapy or had radiotherapy.

Patients with LGM were categorized into three groups. Group LGM 1 – patients examined before the hematologic therapy; group LGM 2 – patients examined 1 month after the therapy; group LGM 3 – patients examined while in permanent remission (1-5 years). The control group included 70 healthy children aged 6–16 years without a history of a systemic disease.

Methods of this study were periodontal examination and screening of salivary lipid peroxidation and enzymatic antioxidants. Periodontal examination was performed in compliance with the guidelines for periodontal screening in children [10]; the complaints, medical history, and clinical criteria (gum color, swelling, bleeding during periodontal probing) were used to make diagnosis of gingivitis or periodontitis in examined children. A periodontal probe was utilised for examination (AEP WHO B; Hu-Friedy). Frequency of gingivitis and periodontitis was evaluated. The children were instructed to clean teeth with a soft brush or a cotton pick.

Saliva samples were collected from children similar to this previously reported Vahabzadeh et al. [11]. The samples were stored frozen at −20°C and were processed as soon as possible to minimize the effects of storage. Clinical unstimulated salivary samples were collected from 40 healthy children and 25 children with LGM. Lipid oxidation was assessed by increasing in the concentration of TBARS during 1,5 hours incubation in iron-ascorbate buffer solution. TBARS was analyzed in the samples by the formation of a stained trimethine complex during the reaction of tiobarbituric acid [12]. Also the activity of antioxidant enzymes – SOD and catalase was studied [12].

The Chi-square test was used to explain the significant differences in periodontal disease frequencies. Categorical variables of salivary indices were analyzed by Student's t-test. Statistical significance level of $p<0,05$ was applied for all calculations.

RESULTS

The children from LGM 1 group showed a significantly higher frequency of periodontal disease as compared with the control group in 6-11 year olds (50,0% vs 17,4%) and 12-15 year olds (80,8% vs 42,8%, $p<0,05$) (Table I). Children from LGM 2 group showed a higher frequency of periodontal disease in 6-11 year olds (60%) and 12-15 year olds (90%) compared with the control group ($p<0,05$) (Table II). Same trend was found in the LGM 3 group (Table III). The frequency of periodontal disease increased in 6-11 years old children over the course of LGM ($p<0,05$). Mostly, gingivitis represented periodontal disease and only 12-16 year olds from LGM 3 group had periodontitis at 16% cases.

Healthy children with periodontal disease had higher TBARS level than counterparts without it (7,9 vs 5,7 umol/l, $p<0,001$). TBARS level in LGM 1 group without periodontal disease (8,01 umol/l) was higher than in healthy children ($p<0,01$) (Table II). This index increased in the LGM 1 group with periodontal disease (9,79 umol/l) as compared to the control group with periodontal disease ($p<0,001$) and LGM 1 group without periodontal disease ($p<0,01$). TBARS level increased over the course of LGM, and was higher in the LGM 2 group with periodontal disease (12,3 umol/l) as compared to the healthy children with periodontal disease ($p<0,001$) and LGM 2 group without periodontal disease (10,1 umol/l, $p<0,01$) (Table III). Similar trend was found in the children from LGM 3 group (Table IV), moreover this index was higher in children without periodontal disease (11,6 umol/l) and with periodontal disease (12,6 umol/l) as compared to the LGM 2 group ($p<0,01$).

Healthy children with periodontal disease had higher SOD activity than counterparts without it (-0,04 vs -0,02 units, $p<0,01$). SOD activity in LGM 1 group without periodontal disease (-0,048 units) was higher than in healthy children ($p<0,01$) (Table II). This index increased in LGM 1 group with periodontal disease (-0,075 units) as compared to the control group with periodontal disease ($p<0,01$) and LGM 1 group without periodontal disease

Table I. Frequency of periodontal disease in children 6-16 years with LGM

Group	Age, years	n	Frequency of periodontal disease	
			n	%
Control	6-11	35	12	17,4±6,1
LGM 1	6-11	20	10	50,0±11,1
				p_1<0,01
LGM 2	6-11	20	12	60,0±10,9
				p_1<0,01 p_2>0,05
LGM 3	6-11	20	16	80,0±8,9
				p_1<0,01 p_2<0,05
Control	12-16	35	15	42,8±6,2
LGM 1	12-16	20	16	80,8±8,9
				p_1<0,01
LGM 2	12-16	20	18	90,0±6,7
				p_1<0,01 p_2>0,05
LGM 3	12-16	25	19	76,0±8,7
				p_1<0,01 p_2>0,05

Note: p_1 – as compared to control group; p_2 – as compared to LGM 1 group

Table II. Lipid perioxidation and enzymatic activity in saliva of LGM 1 group

Index	LGM 1 group, n=20		Control group, n=40	
	Healthy periodontium, n=7	Periodontal disease, n=13	Healthy periodontium, n=25	Periodontal disease, n=15
TBARS umol/l	8,01±0,31	9,79±0,18	5,70±0,18	7,90±0,55
	p_1<0,01 p_2<0,01 p_3<0,01	p_1<0,01 p_2<0,001		p_1<0,001
SOD, activity units	-0,048±0,0045	-0,075±0,0063	-0,02±0,007	-0,04±0,0038
	p_1<0,01 p_2<0,01 p_3<0,01	p_1<0,01 p_2<0,001		p_1<0,001
Catalase, activity units	7,30±0,35	6,72±0,55	8,97±0,24	7,10±0,32
	p_1<0,01 p_2<0,01 p_3<0,01	p_1<0,01 p_2<0,001		p_1<0,001

Note: p_1 – as compared to control group with healthy periodontium, p_2 – as compared to control group with periodontal disease, p_3 – as compared to LGM group with periodontal disease

(p<0,001). SOD activity was higher in the LGM 2 group with periodontal disease (-0,086 units) as compared to the healthy children with periodontal disease (p<0,001) and the LGM 2 group without periodontal disease (-0,059 units, p<0,01) (Table III). Similar trend was found in the children from LGM 3 group (Table IV), however, SOD activity decreased in LGM children without periodontal disease (-0,04 units) and with it (-0,074 units) as compared to parameters of the LGM 2 group (p<0,01).

Healthy children with periodontal disease had lower catalase activity than counterparts without it (7,1 vs 8,97 units, p<0,001). Catalase activity in LGM 1 group without periodontal disease (7,3 units) was lower than in healthy children (p<0,001) (Table II). This index decreased in LGM 1 group with periodontal disease (6,72 units) as compared to the control group with periodontal disease (p<0,01) and LGM 1 group without periodontal disease (p<0,01). Catalase activity was

Table III. Lipid perioxidation and enzymatic activity in saliva of LGM 2 group

Indices	LGM 2 group, n=20		Control group, n=40	
	Healthy periodontium, n=7	Periodontal disease, n=13	Healthy periodontium, n=25	Periodontal disease, n=15
TBARS, umol/l	10,10±0,29	12,30±0,24	5,70±0,18	7,90±0,55
	p_1<0,01 p_2<0,01 p_3<0,01	p_1<0,01 p_2<0,001		
SOD, activity units	-0,059±0,0046	-0,086±0,0078	-0,02±0,002	-0,04±0,0038
	p_1<0,01 p_2<0,01 p_3<0,01	p_1<0,01 p_2<0,001		
Catalase, activity units	5,10±0,45	3,70±0,65	8,97±0,24	7,10±0,32
	p_1<0,01 p_2<0,01 p_3<0,01	p_1< 0,01 p_2 < 0,001		

Note: p_1 – as compared to control group with healthy periodontium, p_2 – as compared to control group with periodontal disease, p_3 – as compared to LGM group with periodontal disease

Table IV. Lipid perioxidation and enzymatic activity in saliva of LGM 3 group

Index	LGM 3 group, n=25		Control group, n=40	
	Healthy periodontium, n=7	Periodontal disease, n=13	Healthy periodontium, n=25	Periodontal disease, n=15
TBARS, umol/l	11,60±0,92 p_1<0,01 p_2<0,01 p_3<0,01 p_4<0,01	12,60±1,21 p_1<0,01 p_2<0,001 p_4<0,01	5,70±0,18	7,90±0,55
SOD, activity units	-0,04±0,006 p_1<0,01 p_2<0,01 p_3<0,01 p_4<0,01	-0,074±0,006 p_1<0,01 p_2<0,001 p_4<0,01	-0,02±0,002	-0,04±0,004
Catalase, activity units	6,70±0,32 p_1<0,01 p_2<0,01 p_3<0,01 p_4<0,01	4,70±0,55 p_1<0,01 p_2<0,01 p_4<0,01	8,97±0,24	7,10±0,32

Note: p_1 – as compared to control group with healthy periodontium, p_2 – as compared to control group with periodontal disease, p_3 – as compared to LGM group with periodontal disease, p_4 – as compared to LGM 2 group.

lower in the LGM 2 group with periodontal disease (5,1 units) as compared to the healthy children with periodontal disease (p<0,001), but higher compared to the LGM 2 group without periodontal disease (3,7 units, p<0,01) (Table III). This parameter increased in the LGM 3 group (p<0,01) (Table IV), however, catalase activity was lower in LGM children without periodontal disease (6,7 units) and with it (4,7 units) as compared to parameters of the LGM 2 group (p<0,01) and the control group (p<0,01).

DISCUSSION

The effect of malignant diseases on the periodontal state has been studied in a lot of papers, however, only one research dealt with periodontal changes in patients with Hodgkin`s lymphoma, showing that Russel periodontal index was insignificantly higher in such patients at the remission phase [5]. In the current study, the children with LGM demonstrated a significantly higher frequency of periodontal disease as compared with the healthy children, which increased over the course of

disease. Results close to these in the presented study were obtained by Kaskova et al. [13] in children with acute lymphoblastic leukemia.

Chronic catarrhal gingivitis in healthy children causes an increase of lipid peroxide oxidation parameters and a decrease in the enzymatic activity of antioxidant protective system in oral fluid [14]. However, periodontopathogenic bacteria develop different factors against peroxidation and some anaerobic oral bacteria, including *Streptococcus* species, make use of superoxide dismutases and other enzyms to metabolize oxygen into less harmful derivatives [15], moreover, cariogenic bacteria developed mechanisms of bacterial acid-tolerance [16].

In this study, TBARS level and SOD activity in the healthy children with periodontal disease, represented mostly by gingivitis, was higher than in the counterparts without periodontal disease ($p<0,01$). Results closed to our dates were obtained by Vinnichenko et al. who showed that SOD in saliva increased in patients with mild periodontitis, but decreased at severe stage of its manifestation [17]. Therefore, increased SOD activity could be explained by adaptation mechanism to periodontal disease development. In this study, catalase activity was lower in the children with periodontal disease ($p<0,01$), that agrees with data of Gharbi et al. [18] who showed that patients with periodontitis exhibited a significant decrease in the activities of catalase.

Genetic variation in oxidative stress genes in tumor cells suggests a possible role for oxidative stress in the risk of non-Hodgkin lymphoma [19]. In this study, TBARS level increased over the course of disease, that probably resulted from the decreased immunity of children with LGM and the cytotoxic effect of chemotherapy or radiotherapy. Children had the highest SOD activity and the lowest catalase activity one month after therapy, however, these parameters improved at the remission phase. Glorieu et al. [20] demonstrated that catalase expression is altered in cancer cells, providing their resistance to peroxidation and cytostatics. Skórska et al. [21] showed that higher SOD in serum could be predictor of survival in patients with cancer. So, continued increase of SOD activity with LGM development might be a sign of the remission. However, Tome et al. [22] claimed that an increase of catalase and SOD in lymphoma cells by chronic oxidative stress exposure results in cells with a chemoresistant phenotype. Thus, a role of oxidative stress in pathogenesis of LGM is complicated and has not studied completely yet.

Moreover, presence of periodontal disease in this study altered lipid peroxidation and antioxidant protection in saliva of the children with LGM. These children had significantly higher TBARS level and SOD activity and lower catalase activity compared to their counterparts without periodontal disease ($p<0,01$).

Sum up, both LGM and periodontal disease altered lipid peroxidation and antioxidant protection in saliva of the children. Future enhanced studies may better explicate mechanisms of antioxidant activity changes in patients with periodontal disease related to LGM. To minimize manifestations of periodontal disease related to LGM and its treatment it might be prospective to recommend medicines which regulate antioxidant activity to improve patient`s quality of life.

CONCLUSIONS

6-11 years old children before hematologic therapy showed a higher frequency of periodontal disease (50,0%), as well as 12-15 year olds (80,8%) compared to the control group (17,4% and 42,8% accordingly, $p<0,05$); morbidity increased during next examinations ($p<0,01$).

TBARS levels were higher in LGM children with periodontal disease before and 1 month after hematologic therapy, and at the remission period (9,79, 12,3 and 12,6 umol/l accordingly) as compared to counterparts without periodontal disease (8,01, 10,1 and 11,6 umol/l accordingly, $p<0,01$) and healthy children with periodontal disease (7,9 umol/l, $p<0,01$). SOD activity was higher in LGM 1-3 groups of children with periodontal disease (-0,075, -0,086, -0,074 units accordingly) as compared to counterparts without periodontal disease (-0,048, -0,059, -0,04 units accordingly, $p<0,01$) and healthy children with periodontal disease (-0,04 units, $p<0,01$). Catalase activity was lower in LGM 1-3 groups of children with periodontal disease (6,72, 5,2 and 6,7 units accordingly) as compared to counterparts without periodontal disease (7,3, 3,7 and 4,7 units accordingly, $p<0,01$) and healthy children with periodontal disease (7,1 units, $p<0,01$).

Both LGM and periodontal disease altered lipid peroxidation and antioxidant protection in saliva of the children.

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