ORIGINAL ARTICLE

Salivary Thromboplastic Activity in Children with Cancer

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Abstract

Thromboplastin is a membrane bound glycoprotein and it is overexpressed in many cancer cells. Chemotherapy induce some alterations in either saliva composition or function leading to oral complications. The aim of this study was to investigate the salivary thromboplastic activity of children with cancer and to compare with healthy children. Saliva samples were taken from 15 children who were diagnosed with lymphoid and solid tumor and from 15 healthy children. In saliva samples thromboplastic activity and total protein levels were determined and SDS polyacrylamide gel electrophoresis was also carried out with these saliva samples. For cytological examination, imprint samples were prepared by applying a drop of saliva on a slide. Salivary thromboplastic activity of children with cancer (CC) was significantly decreased when compared with the healthy children (HC). pH of the saliva in CC was significantly decreased. Salivary flow rate did not significantly change in CC. Saliva total protein level was significantly decreased in CC when compared to HC. In SDS PAGE electrophoresis, some protein bands decreased and some protein bands increased when the two group is compared. In cytologic evaluation of saliva samples, keratinized and dysplastic cells increased in the imprint samples of CC. It should be noted that children receiving chemotherapy encounter major problems in oral and dental health, therefore they need special preventive programs for their oral and dental health.

Keywords: Saliva, thromboplastic activity, pH, cancer, salivary cells

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Introduction

One of the important functions of saliva is to protect the oral cavity. Alteration in either saliva composition or function may cause oral problems. Chemotherapy lead to increased susceptibility to infections, xerostomia, while suppressing the immune system (1). Various mucosal lesions, orophrangeal pain, mucosal infections, hyposalivation, taste disturbances, periodontal problems (gingival overgrowth, gingival lesions, bleeding gums) and caries risk are among the oral complications observed in children under chemothrapy (2).

Many tissues, body fluids and saliva have thromboplastic activity (tissue factor, FIII) (3-7). In the oral cavity, salivary thromboplastin contributes to the barrier function of the oral mucosa by providing hemostasis when the tissue damage occurrs (8). Expression of thromboplastin has been shown in many cancer cells. In recent years, thromboplastin is suggested to lead cancer mechanism by acting through different mechanisms of angiogenesis, tumor growth and metastasis (9-11).

In the present study, salivary flow rate, pH, thromboplastic activity, total protein levels were determined in the saliva samples of the children with cancer and SDS polyacrylamide gel electrophoresis is applied to these saliva samples. The saliva imprint samples were also evaluated cytologically.

Material and Methods

This study was carried out with children who have attended Istanbul University, Faculty of Medicine, Pediatric Hematology – Oncology Department. The parents of the children were informed consent to participate in this study.

Saliva samples were taken from 15 children who were diagnosed with lymphoid and solid tumor and from 15 healthy children. The age range was between 3-17. It has been detected that 10 of the children with cancer have lymphoid cancer (leukemia / lymphoma) and 5 of them have solid tumor. Fasting saliva samples were taken from all children at between 8-11 pm. Mouth of all children were rinsed with distilled water prior to saliva sampling. Saliva collection times and the volume of the saliva were recorded. Saliva pH was tested with the pH paper Merck-pH

indikator papier, Nutralit pH=5.5-9.0). Thromboplastic activity and total protein levels were determined with the methods of Quick (13), and Lowry (14), respectively. SDS polyacrylamide gel electrophoresis was also used on the saliva samples (15).

Thromboplastic activities of saliva samples were evaluated according to Quick's one stage method using normal plasma (13). This was performed by mixing 0.1 ml saliva with 0.1 ml of plasma, with the clotting reaction being started on addition of 0.1 ml of 0.02 M $CaCl_2$. All reagents were brought to the reaction temperature (37 °C) before admixture (13). Total protein content of the saliva samples was determined by the method of Lowry (14). In alkali medium, proteins are reacted with cupper ions than reduced by pholine reactive (phosphomolibydic-phosphotungstic acid). The absorbance of the blue colored product at 500 nm was evaluated. Bovine serum albumin was used as a standard. Total protein levels were expressed as % mg of saliva.

For cytological examination, imprint samples were prepared by applying a drop of saliva on a slide. The slides were first stained 7 minutes with concentrated May Grünwald solution and then 13 minutes with 1/20 diluted Giemsa solution. Thenafter staining slides were washed one minute with distilled water. Dried slides were closed with lamella and were examined under a light microscope (16).

Statistics

The data was evaluated with Unistat 5.0 statistical package programme. Data are presented as mean±standard deviation. The differences between the values of two groups were tested by student *t*-test and Chi-Square test. Differences with p values of 0.05 or less were considered significant.

Results

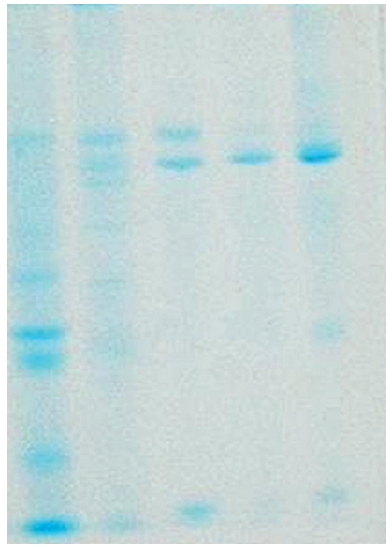
Salivary thromboplastic activity of CC was significantly decreased when compared with the HC ($p<0.05$). Since the clotting time is inversely proportional to the thromboplastic activity, the lengthening of the clotting time is a manifestation of decreased TF activity. pH of the saliva in CC was significantly decreased ($p<0.01$) whereas salivary flow rate did not change in CC ($p>0.1$). Salivary total protein levels were significantly decreased in CC when compared to the HC ($p<0.05$) (Table 1). In SDS PAGE electrophoresis, some protein bands decreased and some protein bands increased when these two groups are compared (Figure 1). In cytologic evaluation of saliva samples, keratinized and dysplastic cells increased in the imprint samples of CC (Figure 2).

Table 1: Salivary pH, flow rate, thromboplastic activity and total protein of all children.

	Healthy Children (n=15) Mean ± SD	Children with Cancer (n=15) Mean ± SD
Age	8,86 ± 2,20	9,71 ± 4,38
Saliva Flow Rate (mL/min)	0,40 ± 0,17	0,41 ± 0,19
Saliva pH	7,13 ± 0,40	6,35 ± 0,66**
Salivary Thromboplastic Activity (sec)	77,47 ± 35,66	134,70 ± 80,01*
Saliva Total Protein (mg/dl)	264,33 ± 76,85	170,39 ± 142,87*

Values are given mean±standard deviation (SD); Student *t* test: *p<0.05 and **<0.01 : significantly important from health children

Figure 1: Saliva SDS polyacrylamide gel electrophoresis



(1: Standard, 2-4: Saliva samples of children with cancer, 5: Saliva sample of healthy children)

Discussion

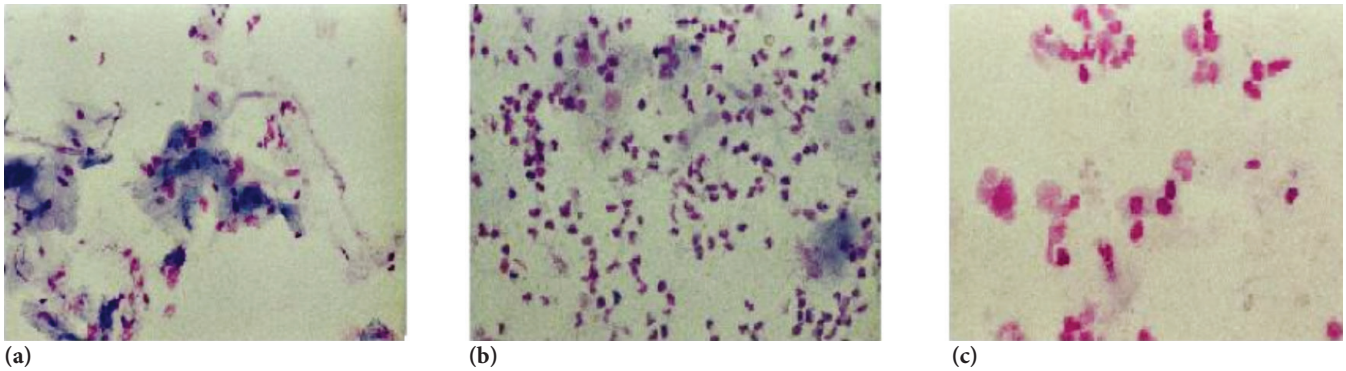
Thromboplastin as a transmembrane protein has various roles in embryogenesis, wound healing, inflammatory responses, tumor growth, metastasis and angiogenesis (12). Cytokines, growth factors, endotoxin, hypoxia conditions, etc. causes the formation of thromboplastin in cells (17, 18). In addition, various tissues and body fluids have been shown to have thromboplastic activity (3-6).

Human saliva triggers coagulation, but the mechanism and physiologic relevance are unknown (19). One of the coagulant factors of human saliva is thromboplastin. In the oral cavity, salivary thromboplastin contributes to the barrier function of the oral mucosa by providing hemostasis when the tissue damage occurrs (3,4,6).

Many studies show the increase of expression of thromboplastin in several types of cancer (9, 12).

In the present study, 5 of the children with cancer was Acute Lymphoblastic Leukemia (ALL) and one of them was Acute Myeloblastic Leukemia (AML). ALL is the most frequent type of cancer seen in the children and its prognosis is the best when compared to other types. AML constitutes 20% of the childhood cancers. Growth can be seen on the gingiva especially in AML and sometimes in ALL because of the infiltration of leukocyte cells. Lesion is red, blue, and sometimes there is bone invasion. In addition to the gingival lesions, fever, bleeding gums, bone and joint pain can be observed (2).

Figure 2: Saliva imprint samples



(a) **(b)** **(c)**

a) Salivary leukocyte cells in healthy children **b)** Salivary leukocyte cell increase in children with cancer **c)** Salivary displastic cells in saliva of children with cancer

Table 2: Cytological evaluation of saliva imprint samples

	Healthy Children (n=15) (%)	Children with Cancer (n=15)(%)	P (Chi-Square)
Epithelial Cells			
(1)	28,6	15,4	p>0,1
(2)	71,4	84,6	
Leukocyte Cells			
(0)	78,6	53,8	p>0,1
(1)	7,1	23,1	
(2)	14,3	23,1	
Displastic Cells			
(0)	0	61,5	P<0,05
(1)	0	38,5	
Bacteria			
(0)	7,1	0	p>0,1
(1)	21,5	53,9	
(2)	71,4	46,1	
Yeast Cells			
(0)	78,6	92,3	p>0,1
(1)	0	7,7	
(2)	21,4	0	
Keratinized Epithelial Cells (0)	85,8	46,1	p<0,05
(1)	7,1	23,1	
(2)	7,1	30,8	

Epithelial Cells	(1): 7-8 cells (normal) (2): 15-20 cells (many)
Leukocyte Cells	(0): No (1): 7-8 cells (normal) (2): 15-20 celsl (many)
Displastic celss	(0): No (1): Present
Bacteria	(0): No (1): few (2): many
Yeast cells	(0): No (1): few (2):many
Keratinized epithelial cells	(0): No (1): few (2) :many

Saliva flow rate and pH are factors that affect the caries activity. It has been suggested that, saliva flow rate below 0,75 mL/min in healthy individuals indicates the susceptibility to dental caries (20). Dissolution of an enamel begins when the saliva pH is under 5.5 (21). In our study, significantly lower saliva pH in CC when compared to HC shows the risk of dental caries for CC. In terms of salivary flow rate, no significant difference was found between the two groups.

present study is the first report that compares the saliva thromboplastic activity in both groups of CC and HC. In several types of cancer tissues, thromboplastin expression has been shown to increase (9,12). Due to the homology of thromboplastin with cytokine receptor of family members, increased expression of thromboplastin from malign and inflammatory cells is not surprising (17).

In the cytological evaluation of saliva imprint samples, keratinized and displastic cells significantly increased in CC. It can be suggested that this increase in keratinized and displastic cells is related with the cause of cancer disease.

The source of thromboplastin found in saliva is salivary cells, vesicles and exosomes (19, 22, 23). In the present study, despite the significantly increased salivary displastic and keratinized cell counts, salivary thromboplastic activity significantly decreased in CC group. In the present study, salivary pH significantly decreased in CC. The chemotherapeutics may be responsible for this pH decrease. Chemotherapy and/or decreased pH may lead to decrease thromboplastic activity by disrupting the three dimensional structure of the thromboplastin. Regardless of its reasoning, this finding may indicate late wound healing in the oral cavity when any damage occurs.

In an another study that investigates the saliva thromboplastic activity of diabetic and healthy children which conducted by the same authors, a parallel increase was found in thromboplastic activity with the number of the cells that are found in the saliva (4).

Tunali et all (5) reported the increased leukocyte cells and increased thromboplastic activity in healthy children after tooth extraction. They concluded that the increased thromboplastic activity support the wound healing process after tooth extraction . There was an increase in saliva thromboplastic activity one hour after the tooth extraction when compared with the thromboplastic activity before extraction.

In conclusion, it should be noted that children receiving chemotherapy encounter major problems in oral and dental health. Therefore they need special preventive programs for their oral and dental health.

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