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Plasma Level of Tissue Factor Pathway Inhibitor in Children With Idiopathic Recurrent Epistaxis (Nosebleeds)

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Previously TFPI has never been studied in childhood nosebleeds. In this study, we determined the free plasma level of TFPI in children with recurrent attacks of idiopathic epistaxis (nosebleeds), during active attacks of nasal bleeding and 24-48 h after treatments (remission state). We investigated 50 children. They were divided into 2 groups. Group 1, 20 children during active attack of nosebleeds. Group 2, 30 children during remission state. All patients enrolled in this study were selected from ENT-Out Patient Clinics Al-Azhar University Hospitals Cairo, Egypt (El-Hossain Hospital). Results were compared to 30 healthy controls age and sex matched. Free plasma TFPI was measured by Enzyme-Linked Immunosorbent Assay (ELISA) in all patients and healthy control child. There was high significant increase of free plasma TFPI levels in children during acute nosebleeds and in remission state (group 1 and group 2) in comparison to healthy control children (group 3) ($p < 0.0001$, $p < 0.0001$), respectively. The highest level of free plasma TFPI was recorded in group 1, followed by children in group 2. In the same time, TFPI level in group 1 of children was highly significantly increased as compared to the level in children in group 2 ($p < 0.001$). As regard gender, there were highly significant increase in free plasma levels of TFPI in both groups (group 1 and 2) as compared to the same gender of the control children (group 3) ($p < 0.001$ and $p < 0.001$), respectively. Comparing hemoglobin levels, there was significant decrease in its level in children in group 1 and II when compared to children in group 3 (control) ($p < 0.01$ and $p < 0.01$). From the present study we concluded that, free plasma level of TFPI is increased in children with idiopathic recurrent nosebleeds, comparable to healthy control children matched for age and sex. The highest level of free TFPI was recorded in children with active attacks of nosebleeds. This high level of TFPI may reflects the degree of endothelial cells dysfunction in this group of children and the state of vascular wall cells in microvasculature of their Little's areas. Further studies are recommended to clarify more the role of the increased free TFPI in pediatric cases with recurrent idiopathic epistaxis and its effect on endothelial cells function as well as vascular wall cells integrity in microvascular circulation.

Key words: Epistaxis, nosebleeds, Tissue Factor Pathway inhibitor (TFPI), idiopathic-children-recurrent

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INTRODUCTION

Epistaxis is defined as acute bleeding from the nostril nasal cavity or nasopharynx. It is well known that, in about 90% of all pediatric epistaxis, no underlying systemic cause, but recurrent episodes are a source of significant distress and anxiety in children, their carers and clinicians. Such episodes of nosebleeds are common cause of morbidity and hospital referral (Stankiewicz, 2004).

Recurrent idiopathic epistaxis in children is repeated, self-limiting nasal bleeding up to the age of 16 years for which no specific cause has been identified. Although it is very common in children, it is rare before the age of two years (Burton and Doree, 2005). In the majority of these children, the bleeding is from the anterior septum and is usually attributed to crusting, vestibulitis and digital trauma (nose picking) (Kubba *et al.*, 2001).

Anterior bleeds are responsible for about 80% of epistaxis however; most (80-90%) are actually idiopathic. They occur at an anastomosis called Kiesselbach's plexus on the lower part of the anterior septum known as Little's area (Pope and Hobbs, 2005). In most cases the bleeding stops spontaneously, but severe or recurrent cases may need hospital treatment (Vaiman *et al.*, 2002). The most common interventions for these cases are cautery of visible vessels and the application of an antiseptic nasal cream to reduce vestibulitis and crusting (Burton and Doree, 2005).

TFPI is a multivalent kunitz-type protease inhibitor of 276 amino acids and approximately 42 kDa that has three domains (Bajaj *et al.*, 2001). It is synthesized mainly by the vascular endothelium (80-85%) and has also been detected in at least four intravascular pools, e.g., bound to the endothelial surface, associated with lipoproteins (10%), carrier-free within the plasma and sequestered in platelets (3%) (Hackeng *et al.*, 2006). TFPI interact with blood coagulation factor VIIa/tissue factor complex and activated factor X via its kunitz-type domains 1 and 2, whereas the K3 domain appears to lack protease inhibitory activity (Piro and Broze, 2004). Furthermore, it plays an important role in inhibiting Tissue Factor (TF)-induced coagulation by factor Xa-dependent pathway of the activated tissue-factor VIIa complex, preventing formation of microvascular thrombosis (Hackeng *et al.*, 2006). A major pool of TFPI is the form associated with the surface of endothelial cells, which is speculated to play an important role in regulating the function of vascular wall cells and modulation of cell proliferation (Kato, 2002). It is cleared from circulation primarily by the liver and the kidney (Van Dreden *et al.*, 2001).

The aim of the present study was to evaluate the free plasma levels of TFPI in childhood idiopathic recurrent nosebleeds (epistaxis), during active bleeding and in

remission state (within 24-48 h after treatment), as it may be used as a marker for endothelial cells dysfunction in these cases or to reflect the state of functioning vascular wall cells in microvasculature circulations.

MATERIALS AND METHODS

The present study included three groups of children. All were enrolled from E.N.T Out-Patient Clinics in Al-Azhar University Hospitals Cairo, Egypt (El-Hossin Hospital) from May to Aug, 2005.

Group 1: Comprised 20 children during active attacks of nosebleeds, they were 9 males and 11 females. Their age range was (4-12) years with a mean (6.9±2.45) years.

Group 2: Comprised 30 children in remission state (within 24-48 h) they were 16 males and 14 females. Their age range (3-11) years and a mean (6.37±2.4) years. They were received medication in the form of vitamin C, cautery and an application of nasal antiseptic cream.

Group 3: Comprised 30 healthy control children. They were 16 males and 14 females. Their age range (3-8) years with a mean (5.5±1.53) years.

Exclusion criteria: Patients older than 16 years, children with any bleeding disorders (coagulopathy) were excluded. Patients with renal insufficiency, liver disease or malignancy, subject on heparin therapy or patients without parental consent, were not enrolled in the present study. Patients with thromboembolic disorders and stroke were also excluded. All patients and controls were subjected to the following:

- Full clinical history, age of onset of epistaxis, its frequency, amount, duration, unilateral or bilateral. Family history of mucocutaneous bleeding, history of anticoagulant drug intake. For assessment severity of bleeding, we used epistaxis scoring system developed by Katsanis *et al.* (1988). Scores of (0-6) were classified as mild, scores of (7-10) were severe.
- Thorough pediatrics examination and, E.N.T evaluation using head-light and anterior rhinoscopy to evaluate the nasal cavity in children with nosebleeds. As well as, to exclude local causes of epistaxis.

A-sampling:

- Three milliliters of venous blood were collected in pyrogen free-tubes (contain Na citrate) citrated blood

with 1:9 ratio (1 Na citrate-9 blood) centrifuged at 3000 rpm for 10 min. Plasma was immediately separated and kept at -20°C until assayed to measure free plasma TFPI.

- Blood samples: Venous blood, for complete blood picture and total platelets count.
- Bleeding time, prothrombin time and activated partial thromboplastine time were done to all patients and controls.
- Complete blood picture using Automated coulter counter T-660
- Free plasma TFPI was measured by immunosorbent assay (ELISA) as follows:

IMUSBIND for assay of total TFPI using a sandwich ELISA employing a rabbit anti-human TFPI native polyclonal antibody as the capture antibody. Specificity of the capture antibody for native, complexed and truncated TFPI was conformed by Westem blot. Analysis visualizing a single band at 34 KD, corresponding to the mobility of intact native TFPI and visualizing a single band at 21 KD, corresponding to the mobility of a truncated form of TFPI. Diluted plasma samples incubate in microtest wells pre-coated with this capture antibody. TFPI is detected using a biotinylated monoclonal antibody specific for the kunitz-domain 1 of TFPI the subsequent binding of the streptavidine conjugated Hoarse Redish Peroxidase (HRP) complete the formation of the antibody enzyme detection complex. The addition of TMB substrate and its subsequent reaction with HRP provides a blue colour. Sensitivity is increased by addition 0.5 M sulphoric acid stop-solution yielding a yellow colour. TFPI levels are determined by measuring sample solution absorbance at 450 nm and comparison against those of a standard curve developed using native TFPI.

Statistical analysis: Statistical analysis was done using Statistical Package for Social Sciences (SPSS) software version 12 (Chicago, USA). Student t-test was applied for comparison of two groups of quantitative data. ANOVA-test used for comparison of more than two parameters. In addition, Spearman's correlation coefficient (r-value) was used to evaluate the correlation between TFPI and others clinical and laboratory parameters. p-value <0.05 considered significant.

RESULTS

Children with active nosebleeds (group 1) and children in group 2 (in remission) had statistically significant decrease in their haemoglobin levels as

Table 1: Clinical and Laboratory data of all patients and controls (Range, Mean±SD)

Variables	Group 1 (nosebleeds) n = 20	Group 2 In remission. n = 30	Group 3 controls n = 30
Age/year	6.9±2.45 (4-12)	6.37±2.4 (3-11)	5.5±1.53 (3-8)
Sex/male	n = 9	16 = n	16 = n
Female	n = 11	14 = n	14 = n
Epistaxis score (%) Severe	18	16	-
Platelets count (10 ⁹ L ⁻¹)	270±64.7 (180-334)	272±60.4 (182-341)	271±65.5 (181-336)
Hemoglobin level (g dL ⁻¹)	10.645±0.571* (9.5-11.5)	10.833±0.663* (9.5-11.9)	12.553±0.6044 (11.5-13.5)
Bleeding time min ⁻¹	6.05±1.1109 (4-8)	5.796±0.823 (4.5-7.3)	4.92±0.9185 (4-6)
Prothrombin time sec ⁻¹	12.275±0.966 (11-14.5)	12.39±0.570 (11.5-13.1)	11.987±0.4183 (11.5-13)
Partial thrombo-plastin time sec ⁻¹	28.825±2.262 (25-33)	30.38±3.157 (26-37)	27.93±0.9185 (25-28)

*: Significant p<0.01 as compared to control group

Table 2: Free plasma levels of TFPI in the three groups of children enrolled in the study with their Genders (Mean, Range±SD)

Items	Group 1 Nosebleeds n = 20	Group 2 In remission n = 30	Group 3 Control n = 30	p-value
Tissue factor				P ₁ >0.12
Pathway inhibitor (TFPI ng mL ⁻¹)	16±5.13 (9-24.2)	7.42±2.52 (2.2-12.1)	2.41±2.3 (0.2-8.7)	P ₂ <0.001 P ₃ <0.001
Males/plasma level of TFPI (ng mL ⁻¹)	14.94±5.81 (9.5-24.2) n = 9	7.34±2.41 (2.2-10.3) n = 16	3.06±2.7 (0.2-8.7) n = 16	P ₂ ≥0.15 P ₃ <0.001
Females/plasma level of TFPI (ng mL ⁻¹)	16.9±4.61 (9-24.5) n = 11	7.5±2.80 (2.4-12.1) n = 14	1.65±1.29 (0.3 3.7) n = 14	P ₁ ≥0.21 P ₂ <0.001 P ₃ <0.001

P1 is p value when group 1 and II and group 3 were compared by ANOVA test in all patients variables; P2 is p-value when group 1 and II were compared to group 3; P3 is p-value when group 1 was compared to group 2

compared to control group (group 3) (p<0.01). As regard to the epistaxis scores in group 1 and 2 no statistically significant difference between them (Table 1 as % severe) because most of patients recording mild score of epistaxis.

In Table 2 the free plasma levels of Tissue Factor Pathway Inhibitor (TFPI) in all studied groups. In group 1 and 2, there were an increase in their plasma levels of free TFPI, which was statistically highly significant as compared to the control group (p<0.0001, p<0.001). Also, male and female patients in the same groups (group 1 and 2) had an increase level of plasma free TFPI, which was statistically highly significant as compared to males and female patients in control group (p<0.0001, p<0.001), respectively. As regard gender in group 1 and 2, there was no statistically significant difference between them. Furthermore, there was statistically significant difference between free TFPI in plasma of children in group 1 (active nosebleeds) when compared to children in group 2 (in remission) (p<0.001) Table 2. The highest level of

TFPI was recorded in group 1 and its gender as compared to children in both groups (group 2 and 3) Table 2. No correlation was found between neither age nor sex in all the enrolled cases or controls in the present study (data not shown).

DISCUSSION

Epistaxis (nosebleeds) is a common childhood symptom that may prompt a referral to a pediatric hematologist or otorhinolaryngologist (Claudio Sandoval *et al.*, 2002) and is usually minor and self-limiting (Burton and Doree, 2005). Nosebleeds affect 30% of children aged 0-5 years, 56% of those aged 6-10 years and 64% of those aged 11-15 years. There is very little in the published literature on recurrent idiopathic epistaxis in childhood and the spontaneous resolution rate is unknown (Kubba *et al.*, 2001).

In the present study, there was significant decrease in hemoglobin level in children with nosebleeds (group 1) and those in remission (group 2) as compared to normal healthy control children (group 3). Commonly anemia may be one of presenting clinical and/or laboratory investigations in children with recurrent epistaxis due to repeated bleeding or severity of bleeding. This agrees with present study and in the work done by Callejo *et al.* (1998), who reported that; anemia is one of the clinical and laboratory manifestation of recurrent attacks of nosebleeds in children. Also, Dai and Jorges (2005) illustrate an atypical presentation of epistaxis with anemia and hypovolaemic shock in a girl 10 months old. Controversially, Claudio *et al.* (2002), reported, no change in hemoglobin level in children attending ENT department with recurrent idiopathic nosebleeds without coagulopathy diseases.

TFPI is expressed by different cell types, such as platelets, monocytes, macrophages and smooth muscle cells, but endothelial cells are thought to represent the principal source of this protein (Morange *et al.*, 2004). TFPI is a 42-kDa protein that consists of three closely linked kunitz domains (Hack, 2000). These domains allow TFPI to function by a unique mechanism. The dynamics of TFPI in the microvasculature are rather complex (Opal and Esmon, 2003) its releases inhibits and prevents microvesicular thrombus formation (Fuster *et al.*, 2005) and administration of exogenous recombinant TFPI has been claimed to protect against intravascular coagulation and venous thrombosis in animals (Huynh *et al.*, 2001). In the present study, free plasma TFPI was significantly elevated in children with idiopathic recurrent epistaxis during acute attacks of bleeding and in between attacks (within 24-48 h) when compared to control healthy children. The

highest levels of free form of TFPI were recorded in the children with active attacks of nosebleeds (group 1). Present suggestion is that, this high level of free TFPI may be due to disturbed blood vessels function and integrity in this Little's area, which reflects the endothelial cells state mainly found in the microcirculation as the main source of this free TFPI is the endothelial cells lining the blood vessels. While the decreased level of it in remission state can be attributed to partially improving in the function of vascular wall cells with vitamin C treatment and/or degradation of this protein in liver and kidney.

Previously TFPI has never been studied in childhood nosebleeds. But many studies have been recorded its level in some diseases, as a risk factor for an occurrence or as a marker of endothelial cell dysfunction. Abdel Gader *et al.* (2005) has reported. A significant elevation of TFPI in children with nephrotic syndrome comparable to healthy control children.

Furthermore, increased levels of TFPI were reported also, in diabetic patients (Leurs *et al.*, 1995) and in patients suffering from cancer or ischemic heart disease (Fuster *et al.*, 2005; Iversen *et al.*, 1998). Meanwhile, decreased level of TFPI value have been found in patients with venous thrombosis or ischemic stroke as well as in coronary circulation of patients with acute coronary syndromes (Dahm *et al.*, 2003; Golino *et al.*, 2003).

In present study, no correlation was found between age or sex of children and their free plasma level of TFPI (not shown). Controversially, in the study done by Duering *et al.* (2004) they; found a negative correlation between level of TFPI and age of the patients. This can be due to small sample of cases enrolled in our study and narrow age range of cases and controls.

Normally, TFPI has very low plasma concentration; about 20% of TFPI circulate in plasma associated with lipoprotein. The majority remains associated with the endothelial surface bound to the cell-surface glycosaminoglycans (Bashir and Bass, 2006). The free portion of TFPI presents high anticoagulant activity and its plasma level strongly correlates with endothelial cell markers (Morange *et al.*, 2004; Pinotti *et al.*, 2005; Blann *et al.*, 2000). So, in the present study idiopathic recurrent nosebleeds can be explained by or attributed to the high levels of plasma free TFPI, assayed in the group of children investigated during acute attacks of nosebleeds. TFPI could be a cause of idiopathic nosebleeds partially due to its high anticoagulant activity of its free portion, as well as due to capillary endothelial dysfunction in microvasculature of Little's area.

CONCLUSIONS

From this study, we concluded that free plasma level of TFPI is increased in children with idiopathic recurrent

nosebleeds, comparable to healthy control children matched for age and sex. The highest levels of free TFPI were recorded in children with active attacks of nosebleeds. Its high level may reflect the degree of endothelial cells dysfunction in this group of children and the state of vascular wall cells in microvasculature of their Little's areas. Further studies and investigations are recommended to clarify more the role of TFPI in pediatric cases with recurrent idiopathic epistaxis and its effect on endothelial cells function as well as vascular wall cells integrity in microvascular circulation.

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