Oxidative stress markers in maxillofacial trauma

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Summary

Local and general effects emerging in organism in consequence of its impact with an aggression evince the intensity of aggression along with the amplitude of the response in the organism.
The *aim* of our study was to observe the disturbances of antioxidant capacity, expressed by Superoxide Dismutase (SOD) and Glutathion Peroxidase (GPx) in children with oral and maxillofacial trauma.
***Material and method*. The study group consisted of 30 politraumatised children (aged 10 to 16 years) with oral and maxillofacial trauma. The patients were hospitalized in the Intensive Care Unit of Constanta Clinical Hospital. The parameters (SOD, GPx) were determined in three different periods of their evolution: T_1 – the first 6 hours after admission, T_2 – after 48 hours and T_3 – 7 days after injury.**
The *results* obtained put into evidence the statistically significant decreased SOD levels at 7 days after injury in comparison with the first two determinations. The levels of GPx were statistically significant decreased after 48 hours and 7 days postinjury in comparison with GPx levels at the time of the first determination (T_1).
***Conclusion*. Monitoring levels of antioxidants in patients with oral and maxillofacial injuries may be useful during therapy for the evolution and prognostic.**

***Keywords*: trauma, maxillofacial, fracture, Superoxide Dismutase, Glutathion Peroxidase.**

Introduction

During the last 25 years, there have been considerable advances in the prevention, diagnosis and management of oral and maxillofacial injuries in children. When compared to adults, the pattern of fractures and frequency of associated injuries are similar but the overall incidence is much lower. Diagnosis is more difficult than in adults and fractures are easily overlooked [1].

Children have a higher surface-to-body volume ratio, metabolic rate, oxygen demand and cardiac output than adults. They also have lower total blood and stroke volumes than adults. Therefore, the risk for hypothermia, hypotension and hypoxia after blood loss is higher in pediatric patients. Trauma to the oral and maxillofacial region

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can involve soft tissues, teeth, and major facial skeletal structures [2,3]. Concomitant injuries involving other areas of the body frequently occur and can complicate overall management.

The local and general effects (hypotension, hypoxia, mass lesion, increased intracranial pressure, ischaemia, free radicals production) which have appeared in the organism after its impact with an aggression, reflect the intensity of the aggression and the amplitude of the organism's response as well [4].

The term "oxidative stress" refers to a shift in the ratio of prooxidant/antioxidant balance. The imbalance can be due to excessive reactive oxygen species production and/or to limited antioxidant defenses [5].

Red blood cells are well equipped to handle intracellular oxidative stress, their membranes are permeable to O_2 – and H_2O_2, and in this way they are important regulators of oxygen reactions occurring in their surroundings. Three types of enzymes detoxify reactive oxygen species: superoxide dismutase (SOD), catalase, gluthatione peroxidase (GPx).

SOD is an enzyme, which protects against toxic effects of oxygen metabolites. The protective effect against reduced oxygen species – generated during the endothelial cell injury of various structures is attributed mainly to the gluthatione metabolism of red blood cells.

Gluthatione peroxidase is an important agent in the inflammatory process. Glutathione (GSH) is a simple tripeptide consisting of the moiety of three nonessential amino acids, L-glutamic acid, L-cysteine and glycine. It plays a key role in the regulation of many enzymatic reactions, participating in several detoxification reactions. Its reduced form represents approximately 90% of total GSH in plasma and exerts its protective role in the metabolism of several toxic agents (participating in oxidative processes) [6].

This article is a pathophysiological study in children with maxillofacial trauma, due to falls or traffic accidents.

Material and method

The study was performed on a test group of 30 politraumatised children with oral and maxillofacial injuries due to falls or traffic accidents, with ages ranging between 10 and 16 years. The patients were admitted to Intensive Care Unit of Constanta Clinical Hospital during December 1999 and July 2002.

In order to select the cases, we consider the following criteria:

1. We chose children admitted in the first 6 hours after accidents, with Glasgow Coma Scale (GCS) between 5 and 13.

2. The lesions consists of:

- dentoalveolar injuries: teeth fractures, teeth avulsion;
- midface fractures, usually resulting from high-impact and/or high velocity forces: zygomatic arc fractures, zygomaticomaxillary complex fractures, nasal fractures, nasoorbital ethmoid complex fractures, and Le Fort fractures type I and II (displaced midface fractures).
- mandibular fractures – the condylar region is the most frequently fractured site, followed by angle and body fractures.

3. Associated injuries were: closed head trauma, temporal bone fractures, extremity fractures, abdominal and thoracic injuries, soft tissue lacerations, without haemorragic shock. The children developed an inflammatory systemic response.

4. The determinations were performed on a seven-day period, reflecting the evolution of the politraumatised children: 19 cases with favourable evolution and 11 cases with unfavourable evolution.

Whole blood superoxide dismutase

(SOD) and gluthation peroxidase (GPx) determinations were conducted in 3 different evolution periods: T_1 – the first 6 hours after admission, T_2 – after 48 hours and T_3 – 7 days after injury.

a) SOD determination technique (using Ransod kit from Randox Laboratories):

This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.T.N.) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction.

$$xanthine \xrightarrow{XOD} uricacid + O_2^{-\cdot}$$

$$I.N.T. \xrightarrow{O_2^{-\cdot}} formazan\ \ dye$$

or

$$O_2^{-\cdot} + O_2^{-\cdot} + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2$$

Sample Preparation

Use heparinised whole blood samples. It is recommended that erythrocytes should be washed four times with 0.9% NaCl solution. Centrifuge 0.5 ml whole blood for 10 minutes at 3000 rpm and then aspirate off the plasma. Then wash erythrocytes four times with 3 ml of 0.9% NaCl solution centrifuging for 10 minutes at 3000 rpm after each wash.

The washed centrifuged erythrocytes should then be made up to 2 ml with cold redistilled water, mixed and left to stand at +4^{o}C for 15 minutes. The lysate is diluted with 0.01 mmol/l phosphate buffer pH = 7.0, so that the % inhibition falls between 30% and 60%. A 25-fold dilution of lysate is recommended for human samples (final dilution factor = 100). Mix, read initial absorbance A_1 after 30 seconds and start timer simultaneously. Read final absorbance A_2 after 3 minutes.

Calculation:

SOD units/ml of whole blood = SOD units/ml from standard curve x dilution factor (100)

$$SODunits / gHb = \frac{SODunits / ml}{gHb / ml}$$

b) GPx determination technique (using Ransel kit from Randox Laboratories):

This method is based on that of Paglia and Valentine. Gluthatione peroxidase (GPx) catalyses the oxidation of Gluthatione (GSH) by cumene hydroperoxide. In the presence of gluthatione reductase (GR) and NADPH, the oxidised gluthatione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured.

Reaction Principle

$$2GSH + ROOH \xrightarrow{GPX} ROH + GSSG + H_2O$$
$$GSSG + NADPH + H^+ \xrightarrow{GR} NADP^+ + 2GSH$$

Sample Preparation

Use heparinized whole blood. It is recommended that Drabkin's reagent is used for dilution. This is due to the presence of peroxidase in human blood, which may give falsely elevated results, and the addition of cyanide serves to inhibit this positive interference. However, dilution of the blood with diluting agent is necessary prior to addition of Drabkin's to convert the gluthatione to the reduced form. This is because, in the oxidized form, cyanide will quickly lead to inactivation. Dilute 0.05 ml heparinized whole blood with 1 ml diluting agent; incubate for 5 minutes and add 1 ml of double strength Drabkin's reagent. Mix well and assay in the normal manner.

Mix, read initial absorbance of sample and reagent blank after one minute and start timer simultaneously. Read again after 1 and 2 minute. Subtract reagent blank value from that of the sample.

Calculation:

Gluthatione peroxidase concentration may be calculated from the following formula:

U/l of haemolysate = 8412 x ΔA 340nm/minute

The result obtained using the RANSEL kit is in units/litre of haemolysate and must be multiplied by the appropriate dilution factor to obtain the result in units/litre of whole blood, thus:

U/l of haemolysate x 41 = U/l of whole blood

For converting to units/gm haemoglobin:

The determinations were performed on LISA 300 PLUS (biochemical analyzer from HYCEL Firm).

$$\frac{\text{U/l whole blood}}{10 \times Hb} = \text{Units GPX/g Hb}$$

In order to assess the significance of the values, we used "t"-Student test on the basis of determination of the arithmetical mean and the standard deviation (SD) of the values.

Results

The results of SOD levels are summarized in *Table 1* and *2*, and represented in *Figure 1*.

The results of GPx levels are summarized in *Table 3* and *4*, and represented in *Figure 2*.

Discussion

The results obtained put into evidence the statistically significant decreased SOD levels ($Pt < 0.05$) at 7 days after injury (T3) in comparison with the first two determinations (T_1 and T_2). Ischaemia, reperfusion, injury and trauma have lead to an accumulation of free fatty acids, which result in the formation of superoxide radicals after reperfusion [7] (*Table 1*).

Under specific therapy the favorable evolution of children with oral and maxillofacial injuries shows statistically significant decreased SOD levels ($Pt < 0.05$) after seven days post injury in comparison with SOD activity in T_1 and T_2. The comparative study based on clinical evolution has shown that the children with unfavourable evolution had statistically significant increased SOD levels in comparison with children with favourable evolution (*Table 2* and *Figure 1*). In all determinations, SOD values are statistically significant increased in the cases with unfavourable evolution in comparison with children with favourable evolution.

Statistically significant decrease of GPx levels ($Pt < 0.05$) after 48 hours and 7 days postinjury in comparison with GPx average at the time of the first determination (T1), reflects the disturbances of antioxidant capacity (Table 3). GPx deficiency directly induces an increase in vascular oxidant stress, with resulting endothelial dysfunction [8].

In all determinations, the children with unfavourable evolution had statistically significant decrease in comparison with children with favourable evolution (*Table 4* and *Figure 2*). We can estimate GPx plasmatic levels as an prognostic indicator of the children with oral and maxillofacial injuries. The lowest levels were found at 7 days after admission, in children with Le Fort fractures type I and II and unfavourable evolution.

Table 1. The statistic analysis of SOD values at admission, after 72 hours and at 7 days

	Admission	**72h**	**7 days**
Average	**2410.93**	**2491.17**	**1842.76**
Standard Deviation	*226.78*	*218.64*	*187.54*
Pt	**-**	**0.08**	**1.9×10^{-15}**
Pt	**-**	**-**	**3.8×10^{-18}**

Table 2. The statistic analysis between SOD values in the three determinations, based on clinical evolution

	Admission		**72 hours**		**7 days**	
	Favourable evolution	Unfavourable evolution	Favourable evolution	Unfavourable evolution	Favourable evolution	Unfavourable evolution
Average	2341.43	2530.99	2426.04	2603.67	1763.44	1979.76
Standard deviation	196.12	234.25	193.53	221.88	169.07	133.96
Pt		0.01		0.02		0.0003

Table 3. The statistic analysis between GPX values in the three determinations, at admission, after 72 hours and at 7 days

	Admission	**72h**	**7 days**
Average	**50.96**	**38.32**	**41.88**
Standard Deviation	*13.19*	*11.52*	*13.77*
Pt		**0.0001**	**0.006**
Pt			**0.13**

Table 4. The statistic analysis between GPx values at the three determinations, based on the clinical evolution

	Test batch - on admission		**Test batch - at 72 hours**		**Test batch - at 7 days**	
	Favourable evolution	Unfavourable evolution	Favourable evolution	Unfavourable evolution	Favourable evolution	Unfavourable evolution
Average	**56.19**	**41.93**	**43.88**	**28.72**	**50.48**	**27.03**
Standard deviation	12.76	8.34	9.46	7.96	8.88	4.99
Pt		**0.0004**		**0.00004**		**2.54×10^{-10}**

Conclusions

Systemic inflammatory response, present in patients with traumatic shock, represents a major source of oxidants. SOD is an enzyme, which protects against toxic effects of oxygen metabolites (tissue reperfusion after a period of ischemia can lead to tissue injury due to free radical generation).

Increased generation of free radicals in traumatized patients is associated with depletion of GSH stores and increased oxidative stress, leading to cellular injury and possibly tissue damage [9].

Monitoring levels of antioxidants in patients with oral and maxillofacial injuries may be useful during therapy for the evolution and prognostic.

Figure 1. Graphic representation of SOD values based on clinical evolution

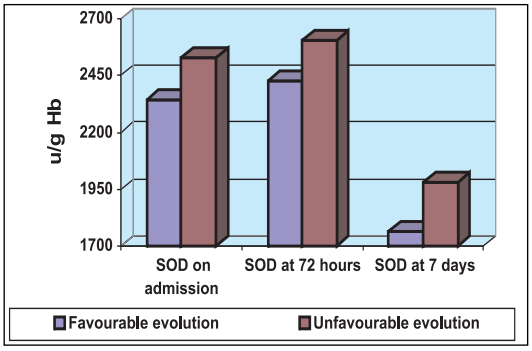
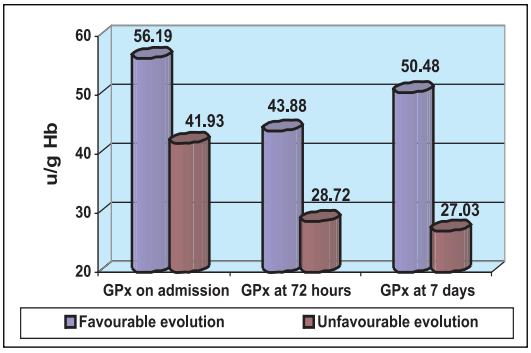


Figure 2. Graphic representation of GPx values based on clinical evolution



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