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Evaluation of Glucagon-Like Peptide 1 (GLP-1) Levels and Oxidative Stress in Preterm Infants

E. Abbasi (PhD)[*1], F. Pishbin (PhD)[2], N. Tahmasebpour Shahsavar (PhD)[3], R. Shahabi (BSc)[3] M. E. Ghafari (MSc)[4], S. Tabatabai (MD)[3], S. Heidari (PhD)[1]

1.Department of Clinical Biochemistry, School of Medicine, HamadanUniversity of Medical Sciences, Hamadan, I.R.Iran.

2.Isfahan Social Security Organization, Isfahan, I.R.Iran.

3.Alzahra Hospital, Tabriz University of Medical Sciences, Tabriz, I.R.Iran.

4.Department of Biostatistics, School of Public Health, Hamadan University of Medical Sciences, Hamadan, I.R.Iran.

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ABSTRACT

Background and Objective: Glucagon-like peptide 1 (GLP-1) has anti-diabetic, antioxidant and anti-inflammatory properties that change under different conditions. This peptide is known as a therapeutic target in diabetes and obesity. The aim of this study was to investigate the relationship between GLP-1 and oxidative stress in preterm infants.

Methods: In this case-control study, 40 term and preterm infants (gestational age less than 37 weeks) were used. GLP-1 concentration was measured by ELISA. Total antioxidant capacity (TAC), total oxidant status (TOS) and uric acid concentration were measured.

Findings: Serum GLP-1 level in preterm infants was significantly higher than term infants (p=0.003). Serum TAC level in preterm infants (973.16±235.53) was significantly lower than term infants (837.00±218.97) (p=0.006). Serum TOS level in preterm infants (65.46±33.52) was significantly higher than term infants (31.50±14.62) (p<0.001). Uric acid levels in preterm infants (5.83±1.73) were statistically higher than term infants (5.13±0.90) (p=0.02). Serum GLP-1 level in preterm infants was negatively and significantly correlated with TAC (p<0.001, r=-0.674) and positively and significantly correlated with TOS (p<0.001, r=0.754) and uric acid (p<0.001, r=0.633).

Conclusion: The results showed that the levels of GLP-1, TOS and uric acid in preterm infants were higher than term infants and GLP-1 had a negative correlation with TAC capacity and a positive correlation with TOS and uric acid in these infants.

Keywords: *Infant, Glucagon-Like Peptide 1 (GLP-1), Oxidative Stress, Uric Acid.*

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***Corresponding Author: E. Abbasi (PhD)**
Address: Department of Clinical Biochemistry, HamadanUniversity of Medical Sciences, Hamadan, I.R.Iran.
Tel: +98 (81) 32520182**. E-mail:** 7abbasi@gmail.com

Introduction

A preterm infant is a live infant born before 37 weeks. It is estimated that about 15 million preterm infants are born worldwide each year. In other words, about 1 in 10 infants, and about one million infants die from preterm birth problems (1). Various studies have shown that in infants, the oxidant-antioxidant balance is regulated in a narrow range. But in preterm infants, this balance progresses toward increased oxidative stress (2). Oxidative stress occurs as a result of an imbalance between the antioxidant defense system and the production of free radicals. Oxidative stress in pregnancy is caused by maternal toxicity as well as delayed intrauterine growth. With increasing oxidative stress in the fetus, the degradation of proteins, fats and carbohydrates increases (3, 4). During pregnancy, increased oxidative stress in the placenta and chorionic villi causes the transfer of free radicals into the mother's bloodstream (5). The use of antioxidant compounds plays an important role in the elimination of free radicals (6-8). Incretins, especially Glucagon-like peptide 1 (GLP-1), are among the compounds that have recently been proven to have antioxidant effects (9). Elevated uric acid levels have been reported to be associated with high blood pressure, low birth weight, and low gestational age (10).

GLP-1 is a type of incretin (secreted from the intestine) whose important antioxidant role through the glucose homeostasis pathway has been proven (11). Incretins are secreted by intestinal cells in response to food intake and play an important role in glucose metabolism (12). In general, incretins stimulate insulin secretion, inhibit glucagon secretion, slow gastric emptying, and induce satiety (13). GLP-1 is secreted from L cells in the ileum of the small intestine and colon in response to the entry of nutrients into the intestine and an increase in blood glucose (14). Today, drugs that affect GLP-1 are used in the treatment of obesity and diabetes (11). Since the serum level of GLP-1 and the amount of oxidative stress in preterm infants compared to term infants have not been studied so far, the relationship between serum levels of this hormone and oxidative stress in infants was investigated in this study.

Methods

This case-control study was approved by the ethics committee of Hamadan University of Medical Sciences with the code IR.UMSHA.REC.1397.293 and conducted among 40 infants born in Al-Zahra Hospital in Tabriz in two groups including 20 preterm infants (by cesarean section) with intrauterine age of less than 35 weeks who were admitted to the Neonatal Intensive Care Unit (NIUC) and the control included 20 infants aged 37-42 weeks (13 infants born by cesarean section and 7 infants born by vaginal delivery) who were not hospitalized. Gestational age was determined by calculating the mother's last menstrual period (LMP) and fetal ultrasound findings. The type of delivery and Apgar were obtained from patients' files.

Inclusion criteria included preterm infants (35 weeks and younger) admitted to the neonatal intensive care unit and term infants aged 37-42 weeks. The exclusion criteria included the presence of any infectious diseases, physical abnormalities, congenital metabolic disorders and being fed with any type of milk before blood sampling. Moreover, infants with an Apgar score of less than 7 or infants whose mother was diabetic or had hormonal disorders were excluded from the study. Prior to the collection of samples and demographic data, informed written consent was obtained from all parents of the infants. Neonatal demographic information was collected through a questionnaire and the information in the files. About one milliliter of venous blood was collected from infants in both groups before any breastfeeding and immediately sent to the laboratory for measurement of total antioxidants, total oxidants, and uric acid. Samples were coagulated at room temperature for half an hour and then isolated using serum centrifugation. Serum uric acid

concentration was measured on the same day and serum residues were stored in the freezer at -80 °C to determine the concentration of other factors. Oxidative stress status parameters including Total Antioxidant Capacity (TAC) and Total Oxidant Status (TOS) were measured manually by a spectrophotometer (Bel, Italy) (15).

TAC was measured by FRAP (Ferric Reducing Antioxidant Power) method. In this method, antioxidant compounds reduce the formation of a blue-purple complex by reducing iron ions. The FRAP method is based on the ability of the sample to reduce ferric ions. The regeneration process is performed in the presence of a substance called TPTZ (Tripyridyl-S-triazine, Sigma-Aldrich). The Fe^{2+}-TPTZ complex has a maximum absorption of 593 nm. TAC levels were reported in nmol/ml (16, 17). Total oxidative capacity (TOS) was measured by oxidation of ferrous iron to ferric under moderate acidity using xylenol orange dye. TOS was reported in nmol/ml (18). Uric acid concentration was measured in both groups by Pars Test Kit and by Electra Analyzer (Vital Scientific NV, DIERN, Netherland). Serum concentration of GLP-1 was also determined by ELISA (Bioassay kit, Germany) and ELISA reader (Sunrise, Austria).

SPSS 20 and T-test and Pearson tests were used to analyze the results. GraphPad Software (San Diego-USA) was used to draw graphs and $p<0.05$ was considered significant.

Results

In the group of preterm infants, there were 8 boys and 12 girls (8/12) and in the group of term infants, there were 10 boys and 10 girls (10/10). The weight of preterm infants (1.74±0.73 kg) was significantly ($p<0.001$) lower than the weight of term infants (3.26±0.53 kg). Fetal age in preterm infants (30.41±3.86) was much lower than term infants (38.43±0.90) ($p<0.001$). The level of TAC in term infants was significantly higher than preterm infants ($p<0.001$). The level of TOS in term infants was significantly higher than term infants ($p=0.006$). Uric acid levels were higher in preterm infants compared to term infants (Table 1).

Statistical analysis shows a significant difference between this factor in the two groups. Comparison of the mean of GLP-1 in the group of preterm and term infants showed that the serum level of GLP-1 in preterm infants was significantly higher than term infants ($p=0.003$) (Figure 1).

The results of Pearson test showed that there was a positive and significant relationship between GLP-1 and uric acid ($p<0.001$, $r=0.633$) and TOS ($p<0.001$, $r=0.754$), but GLP-1 and TAC were negatively correlated ($p<0.001$, $r=-0.674$) (Table 2).

Table 1. Indices of oxidative stress and uric acid in infants

Groups / Variables	Term infants (n=20) Mean±SD	Preterm infants (n=20) Mean±SD	p-value
Uric acid (mg/dl)	5.13±0.90	5.83±1.73	0.02
Total oxidant status (μmol/ml)	31.50±14.62	65.46±33.52	<0.001
Total antioxidant capacity (μmol/ml)	973.16±235.53	837.00±218.97	0.006

The results are shown as Mean±SD.

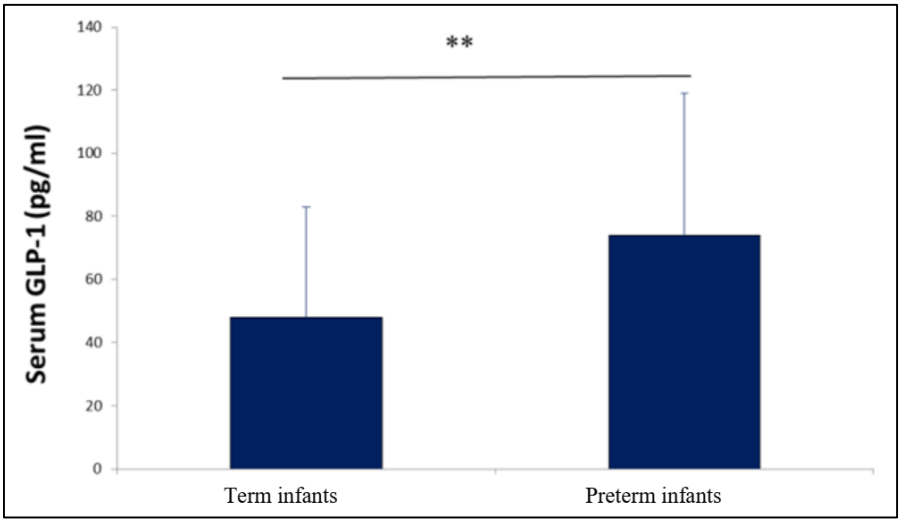

Figure 1. Comparison of GLP-1 concentrations in the serum of preterm and term infants.
The results are shown as Mean±SD.

Table 2. Evaluation of the relationship between serum GLP-1 concentration and oxidative stress indices in preterm infants

Variable	Serum concentration of GLP-1 (pg/ml)	
	r	p-value
Uric acid (mg/dl)	0.633	<0.001
Total oxidant status (μmol/ml)	0.754	<0.001
Total antioxidant capacity (μmol/ml)	-0.674	<0.001

Discussion

The results of this study showed that serum TAC levels in preterm infants were significantly lower than term infants and serum TOS and GLP-1 levels in these infants were higher than infants. Recent studies have shown that in preterm infants, the rate of oxidative stress increases and antioxidant defense decreases, which is involved in various diseases (19). GLP-1 is involved as an antioxidant defense barrier against oxidative stress damage in these infants (20). In this study, the mean serum level of GLP-1, as one of the important intestinal hormones that has an antioxidant role, was measured in preterm and term infants. The results of the present study showed that the mean serum level of GLP-1 in preterm infants was significantly higher than the mean serum level of GLP-1 in term infants. The results of the present study are in line with the study conducted by Shoji et al. The results of this study showed that serum levels of GLP-1 and insulin were higher in preterm infants born before 30 weeks compared to infants born after 30 weeks (20).

The results of this study showed that serum uric acid level in preterm infants was significantly higher than term infants and there was a significant negative correlation between serum uric acid level and gestational age and neonatal weight, and there was a positive correlation with serum GLP-1 level. Consistent with our study, in the only study performed in this area, uric acid levels in the urine of preterm infants were higher than term infants. There was also an inverse relationship between uric acid and gestational age and infant weight. Due to the fact that the concentration of urinary excretion is directly related to their serum concentration, it seems that the serum concentration of uric acid in premature infants is higher than term

infants (21). Another study on neonatal umbilical cord samples showed that uric acid levels in neonatal umbilical cord were higher than maternal serum and increased in the first 24 hours after birth (22). Serum levels of TAC in preterm infants were significantly lower than term infants and serum levels of TOS in these infants were higher than term infants. These results are in line with studies by Abdel Ghany et al. They provided evidence that serum levels of oxidative stress factors were significantly higher in 100 preterm infants compared with the same number of term infants (23). In this study, uric acid levels were higher in preterm infants. Abnormal uric acid has been reported to be positively associated with blood pressure and negatively related to birth weight and gestational age. Therefore, we hypothesized that preterm infants had higher uric acid than term infants. The results of this study are in line with the study of Washburn et al. (10), which showed that uric acid levels are higher in preterm infants.

The results of Pearson correlation showed that serum level of GLP-1 in preterm infants was negatively correlated with TAC and positively correlated with TOS. Given that the relationship between serum GLP-1 concentration and oxidative stress indices in preterm infants has not yet been precisely determined, one of the reasons for the increase in serum GLP-1 concentration in preterm infants is probably the antioxidant role of GLP-1. Tomas et al. showed that GLP-1 inhibits glucose synthesis and oxidative stress (24). Oxidative stress causes insulin dysfunction and eventually leads to diabetes (25). In this study, neonatal weight was studied as one of the important and involved factors in neonatal growth and development. Premature infants were significantly lighter-weight compared to term infants. Studies show that weighing less than 2,500 grams (the result of preterm delivery or intrauterine growth restriction) is a major indicator of complications in infancy (26).

The results of this study showed that the serum level of GLP-1 in preterm infants was significantly higher than term infants. Serum levels of TAC were significantly lower in preterm infants and serum uric acid and TOS levels were higher in these infants. The results of Pearson correlation showed that serum uric acid and TOS levels were positively correlated with serum levels of GLP-1 in preterm infants and serum TAC levels were negatively correlated with serum levels of GLP-1 in preterm infants.

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