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ABNORMAL PHENYLALANINE-TYROSINE METABOLISM MAY PLAY A ROLE IN THE DECREASE OF HAEMOGLOBIN IN HEALTHY PREGNANCY: A COHORT STUDY

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ABSTRACT

Background: Hyperfiltration and lowering of haemoglobin level are documented changes in physiological pregnancy. Serum level of erythropoietin increases in pregnancy and erythropoietin-resistance develops which may be contributed by elevated levels of hydroxil free radical–derived, pathological products of Phenylalanine as meta- and ortho-tyrosine, in contrast to the physiological para-tyrosine-. **Population and methods:** Physiological pregnant women (n=23) and healthy, non-pregnant women (n=26) were enrolled. In the pregnant group blood and urine samples were taken at weeks 12, 24, 36 and at delivery. We determined the concentrations of Phe and the three Tyr isoforms using reverse-phase high performance liquid chromatography. The time kinetics of these and their association with hematopoiesis were studied. Tendencies of p-, m-, o-Tyr and Phe levels, as well as erythropoietin and hematopoiesis parameters were determined. **Results:** In the early pregnancy a decreased serum level of p-Tyr developed ($p<0.05$). In the progression of the pregnancy, p-, m-, o-Tyr and Phe levels showed an increasing tendency ($p<0.05$). Serum levels of m- and o-Tyr and their ratios with Phe and p-Tyr correlated inversely with ($p<0.05$), and were independent predictors of haemoglobin levels ($p<0.05$). Urinary levels of o-Tyr and its ratios with Phe and p-Tyr correlated with and were predictors of red cell distribution width ($p<0.05$). We observed decreased serum haemoglobin and elevated serum erythropoietin levels, suggesting erythropoietin resistance. **Conclusions:** Elevated levels of pathological Tyr isoforms and decreased concentration of p-Tyr are associated with hematopoiesis in physiological pregnancy. Lowered level of p-Tyr can be the result of increased excretion or elevated consumption. We propose that pathological tyrosines may lead to disturbed erythropoiesis.

KEYWORDS*:* tyrosine, gestational anaemia, EPO-resistance, erythropoiesis, pregnancy.

INTRODUCTION

Pregnancy is a state of remarkable metabolic, biochemical, physiological, hematological and immunological changes, and affects all aspects of kidney physiology. Besides anatomic changes, there is a significant volume expansion and vasodilation in the systemic and kidney circulation. Hemodynamic changes may be provoked by maternal hormones. There is an upregulation of the renin-angiotensin-aldosterone system (RAAS) in normal pregnancy due to the extrarenal production of renin in the maternal ovaries and the decidua, which results in a continuous rise in aldosterone levels reaching 3- to 6-fold higher range than that of normal in the third trimester (the upper limit of normal in the third trimester).[1] This results in a 30% to 50% gain in blood volume. Renal plasma flow increases up to 80% compared to nonpregnant women.[2]

Many of the vascular dysfunctional effects of the RAAS are mediated through angiotensin II and aldosterone by the activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzymatic complex resulting in the activation of the NADPH oxidase enzyme which in turn triggers the production of superoxyde free radicals, leading to oxidative stress.[3]

Christensen and colleagues demonstrated up to 30% enlargement of kidneys in healthy pregnant women, which could be attributed to increased kidney vascular and interstitial volume rather than dilatation of the kidney pelvis.[4] The kidney pelvis and calyceal systems dilate during pregnancy due to mechanical compression of the ureters especially the right ureter.[5] Elevated progesterone levels in pregnancy can possibly cause reduced ureteral tone, peristalsis and contraction pressure

contributing to the above mentioned changes. [6] There is a greater incidence of right sided hydronephrosis due to anatomic causes.

A significant rise in the glomerular filtration rate (GFR) is one of the first renal changes during gestation.[1] Several studies suggest a progressive rise in GFR in pregnancy leading to a 40% to 50% increased filtration rate compared to prepregnancy levels.[7]

Anaemia is a common complication during pregnancy and often is a substantial contributor to poor pregnancy outcomes. The World Health Organization (WHO) reports that 23% of pregnant women from the developed countries are anaemic, while according to the "Nutrition Impact Model Study's 2011 estimates" the worldwide prevalence of anaemia in pregnant women was 38%.[8]

Iron deficiency is supposed to be the most common cause of anaemia during pregnancy, however erythropoietin has a key role in the regulation of erythropoiesis, i.e. the process of red blood cells production in bone marrow.

The growth and survival of the erythroid progenitor cells, and the red blood cell production rate is mainly determined by the serum erythropoietin (EPO) concentration, which is inversely related to oxygen availability. The production of this 30,400-dalton glycoprotein hormone depends on the transcriptional activity of the EPO gene in the kidneys. The EPO gene expression increases as a consequence of lowered local oxygen tensions, providing a feedback loop which controls erythropoiesis.[9]

In adults EPO is mainly released in the renal cortical interstitium by type I renal peritubular cells. Normal human serum EPO-concentration when determined with radioimmunological method ranges from 10 to 30 mU/mL, which parallels to 2-7 pmol/L.[9]

Normal range of EPO-concentration in pregnant women has not been determined, however there is an increase in the levels of maternal EPO during gestation due to an activation in the biosynthesis of the hormone in the maternal kidney and an additional site of the hormone production, the trophoblast cell of the human placenta.[10] Oxidative stress is defined as an imbalance between the prooxidant system and the antioxidant defense, which leads to an increased production of free radicals as e.g. hydroxyl free radical (HO•), resulting in direct tissue damage and adversely affecting functional integrity.[11]

In oxidative stress, the conversion of phenylalanine (Phe) to three different tyrosine (Tyr) isomers, i.e. para-, meta-, and ortho-tyrosine (p-Tyr, m-Tyr, o-Tyr) is increased due to hydroxyl free radicals, while the physiological isoform p-Tyr is produced by the Phe hydroxylase enzyme as well. The two other non-physiological isoforms (m-Tyr and o-Tyr) on the other hand are present in very low concentrations under physiological circumstances.[12] Therefore the detection of these abnormal isoforms is considered to be valuable procedure to investigate of free radical derived oxidative damage,[13]

It was shown that elevated levels of oxidative stress contribute to the development of hormone resistances, such as insulin- or EPO- resistance.[14]

According to previous observations made by our study team, the ratio of abnormal over physiological Tyr levels was shown to be an independent predictor of hyporesponsiveness to erythropoiesis-stimulating agents (ESA).[15]

The aim of the present study was to demonstrate that during the physiological pregnancy, the higher production of Tyr isomers could be a component of gestational anaemia by causing hyporesponsiveness to EPO.

PATIENTS AND METHODS

The aim of our study was to show the impact of altered levels of phenylalanine metabolites on erythropoiesis in pregnancy.

Twenty three healthy pregnant women have been enrolled in the study, who gave their written informed consent to having blood drawn for hematologic tests and giving blood and urine samples for the evaluation of Phe, p-Tyr and the hydroxyl free radical markers m-, o-Tyr while undergoing routine antenatal and obstetrical care. Patients with ongoing infections, malignant diseases, diabetes, hypertension and autoimmune disorders requiring treatment could not be enrolled in the study. These patients were selected from our obstetrics and gynaecology unit (Somogy County Kaposi Mór Teaching Hospital, Department of Obstetrics and Gynaecology). Clinical characteristics at 12 weeks of gestation as well as baseline data of red blood cell indices are outlined in Table 1.

Table 1. Characteristics of enrolled patients at 12 weeks of gestation

n	23
Age (years)	29 ± 5
Gravidity	2 ± 1
Parity	1 ± 1
Weight (kg)	66 ± 16
Blood glucose level (mmol/l)	4.1 ± 0.6
eGFR (ml/min/1.73m^2)	158 ±46
Anaemic patients (Hb < 125 g/l)	6 (26.1%)
RBC (T/l)	4.2 ± 0.3
Hb (g/l)	128 ± 7
Htc (%)	37.4 ± 2.3
MCV (fl)	89 ± 4
MCH (pg)	31 ± 1
MCHC (g/l)	344 ± 7
RDW-SD (fl)	43 ± 3

Note, that values of MCV, MCH, MCHC of the anemic patients were also in the reference ranges. Abbreviations: eGFR, estimated glomerular filtration rate; RBC, red blood cell count; Hb, haemoglobin; Htc, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW-SD, red cell distribution width - standard deviation.

The control group consisted of 26 healthy non-pregnant volunteers who also gave informed consent and were involved at the University of Pécs 2nd Department of Medicine and Nephrology Center, (Control, median age 55 years, data not represented in the table). Patients with acute infections, malignancy, and active autoimmune disease failed to meet the enrollment criteria. All results were corrected for age.

In the pregnant group samples were drawn three times during gestation, at weeks 12, 24, 36 and at delivery, via vein puncture into tubes containing clot activator and also midstream urine samples into containers. All blood samples were obtained during routine antenatal visits and on the day of delivery, before the childbirth. Routine analytical procedures were performed using standard laboratory techniques in the Main Laboratory of the Somogy County Kaposi Mór Teaching Hospital.

Serum samples were obtained by centrifugation and separated into 1.5 ml Eppendorf microcentrifuge tubes for freezing. Serum and urine samples were stored at -20ºC until transportation and at -80ºC pending further processing and analysis.

High performance liquid chromatography (HPLC) was performed in the laboratory of the 2nd Department of Medicine and Nephrological Center, University of Pécs. During the procedure after adding 125 µl trichloroacetic acid (TCA) to 500 µl serum/urine the specimen was incubated on ice for 30 minutes, which was followed by a centrifugation in order to separate the precipitate. The supernatant was filtered using a syringe filter (0.2 µm) before further analysis. For the measurement of p-, m-, o-Tyr, and Phe levels we used reverse phase-HPLC (C18 silica column, 250×4 mm) with fluorescence detection (λEX = 275 nm; λEM =305 nm for the Tyr isoforms and λEX = 258 nm; λEM =288 nm for Phe). An external standard was used to detemine concentrations. Details of the method have been described previously.[15]

Quantitative measurement of erythropoietin was performed in the Department of Laboratory Medicine, University of Pécs, using Siemens Immulite 2000 EPO analyzer, which is a solid-phase, enzyme-labeled chemiluminescent immunometric assay.

Statistical analysis of the data has been performed with the non-parametric Mann-Whitney U test, Spearman correlation, Jonckheere-Terpstra test for linear trend, and multivariate linear regression analysis.

Ethics approval and consent to participate

I confirm that the study has been approved by the institutional and research ethics committee of the Somogy County Kaposi Mór Teaching Hospital 06.02.2012. Informed consent was obtained from all individual participants included in the study.

RESULTS AND DISCUSSION

Characteristics of enrolled pregnant women at 12 weeks of gestation are outlined in Table 1. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width –standard deviation (RDW-SD) were within the normal reference range throughout the pregnancy (reference ranges were as follows: MCV, 80-96 fl; MCH, 28-35 pg; MCHC, 320-360 g/l; RDW-SD, 38-50 fl.) suggesting normal iron homeostasis, however the estimated glomerular filtration rate (eGFR) levels were increased in all cases.

Estimated glomerular filtration rate

The eGFR remained abnormally increased throughout the gestation reaching a peak in the third trimester followed by a moderate decrease in term pregnancy. Despite the remarkable increase in eGFR from the first trimester, the rise did not reach the level of significance when using age-adjusted analysis.

Results obtained in serum

Levels of serum phenylalanine (Phe) and tyrosine (Tyr) isomers.

Figure 1 demonstrates the changes in Phe and Tyr isomer levels during gestation. Serum p-Tyr levels were significantly lower at all timepoints in the gestational group ($p < 0.001$) after a remarkable decrease in the first trimester followed by a slow growth in mid pregnancy and returned to almost non-pregnant levels by the time of delivery (Figure 1, Panel a).

There is an initial significant lessening in the serum levels of Phe at 12 and 24 weeks of gestation, followed by rising serum levels in the third trimester resulting in a significant elevation of Phe at term (Figure 1, Panel b).

We found decreased serum level of the o-Tyr at week 24 and an increasing tendency throughout the whole pregnancy (Figure 1, Panel c).

Serum m-Tyr levels were increased at week 24, and we found a significant increasing tendency during the pregnancy (Figure 1, Panel d).

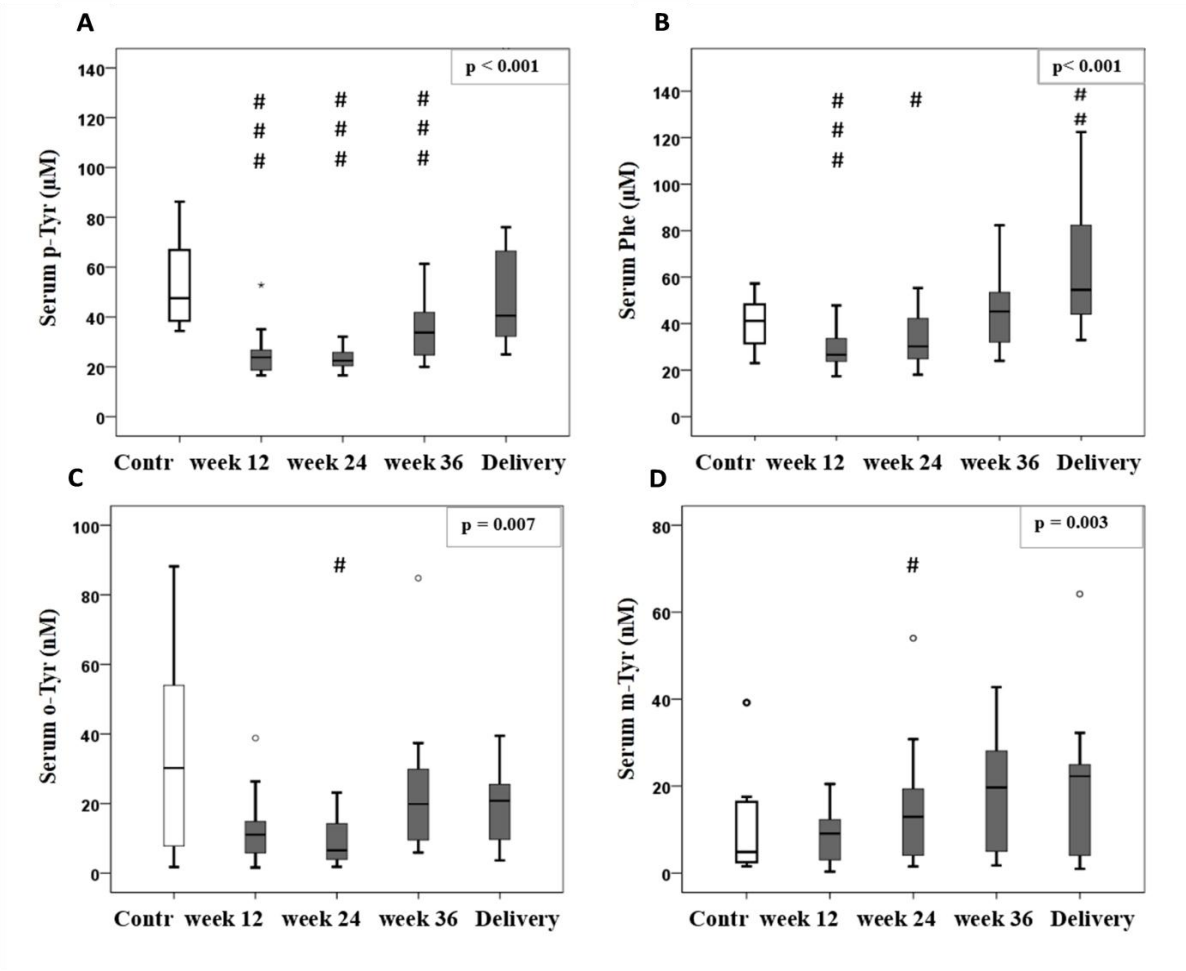

Figure 1: Levels of serum para-tyrosine (A), phenylalanine (B), ortho-tyrosine (C) and meta-tyrosine (D) in non-pregnant controls and at 12, 24, 36 weeks of gestation and at delivery
The p value in the inserted boxes of each panel represents the result of the Jonckheere-Terpstra test for linear trend.
(#, p<0.05; ##, p<0.01; ###, p<0.001 vs. Contr)
p-Tyr, para-tyrosine; Phe, phenylalanine; o-Tyr, ortho-tyrosine; m-Tyr, meta-Tyrosine; Contr, controls;

Serum para (p)-, ortho (o)-, meta (m)-, and o- + m-Tyr/Phe ratios

We calculated the different Tyr to Phe ratios, as well. As mentioned above, in pregnant women there is a significant decrease in serum p-Tyr levels and also a considerable but lower grade reduction in serum Phe amounts, resulting in significantly lower p-Tyr to Phe ratios compared to the control group, as illustrated in Figure 2, Panel a.

The previously observed rise in serum m-Tyr and decline in Phe levels lead to significantly higher m-Tyr to Phe ratio at week 24 and 36 (Figure 2, Panel b), while there was no significant difference in o-Tyr/Phe ratios between pregnants and control subjects, as it can be seen in Figure 2, Panel c.

Figure 2, Panel d demonstrates, that meta- + o-Tyr/Phe ratio showed an increasing tendency during the pregnancy, and the values of pregnant women were significantly higher from the 24th week, than that of control persons.

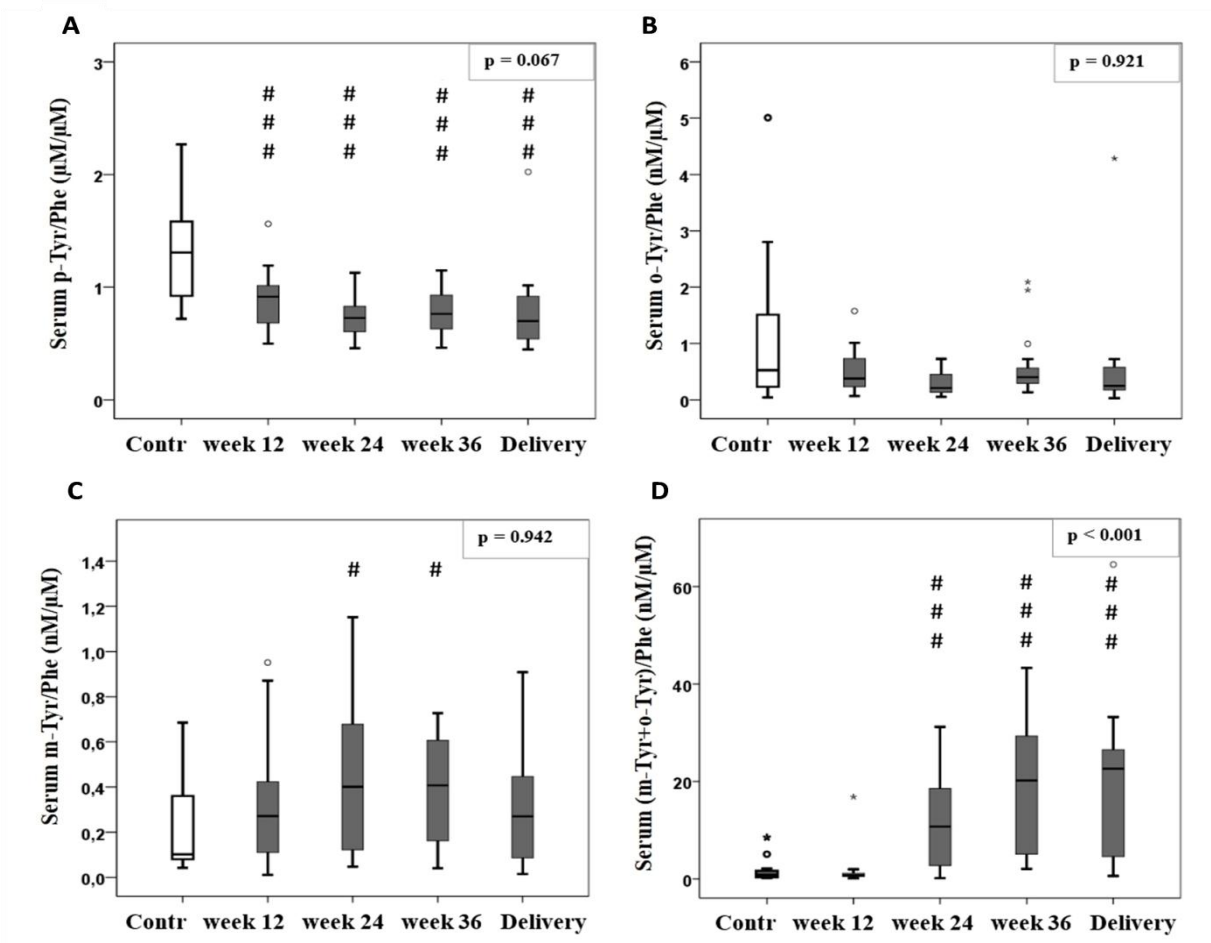

Figure 2: Serum para-tyrosine/phenylalanine (A), ortho-tyrosine/phenylalanine (B) meta-tyrosine/phenylalanine (C) and (meta-tyrosine + ortho-tyrosine)/phenylalanine (D) ratios in non-pregnant controls and at 12, 24, 36 weeks of gestation and at delivery
The p value in the inserted boxes of each panel represents the result of the Jonckheere-Terpstra test for linear trend
(#, p<0.05; ##, p<0.01; ###, p<0.001 vs. Contr)
p-Tyr, para-tyrosine; Phe, phenylalanine; o-Tyr, ortho-tyrosine; m-Tyr, meta-Tyrosine; Contr, controls;

We found, that there was no significant difference in the serum o-Tyr/p-Tyr ratios between pregnant women and the control group, however as a result of elevated serum m-Tyr levels and significantly lower p-Tyr values, the m-Tyr/p-Tyr ratios were higher than in the control subjects, especially in the second and third trimester.

Results obtained in the urine

Levels of urinary Phe and Tyr isomers

We detected a gradually growing rate of urinary excretion of both p-Tyr and Phe during pregnancy, contributing probably to the lower serum levels mentioned above.

Urinary o-Tyr excretion showed a non-significant (p=0.051) decreasing tendency throughout the pregnancy.

The detected changes in the levels of urinary m-Tyr did not achieve the level of significance.

Urinary p-, o-, and m-Tyr/Phe ratios

Urinary p- (Figure 3, Panel a) and o-Tyr/Phe ratios (Figure 3, Panel b) showed a significantly decreasing tendency during pregnancy but they were not significantly different compared to the control group. Urinary m-Tyr/Phe ratios were significantly lower in all trimesters than in the control group. (Figure 3, Panel c). As a result the (m- + o-Tyr)/Phe ratio also showed a decreasing tendency during the pregnancy. (Figure 3, Panel d).

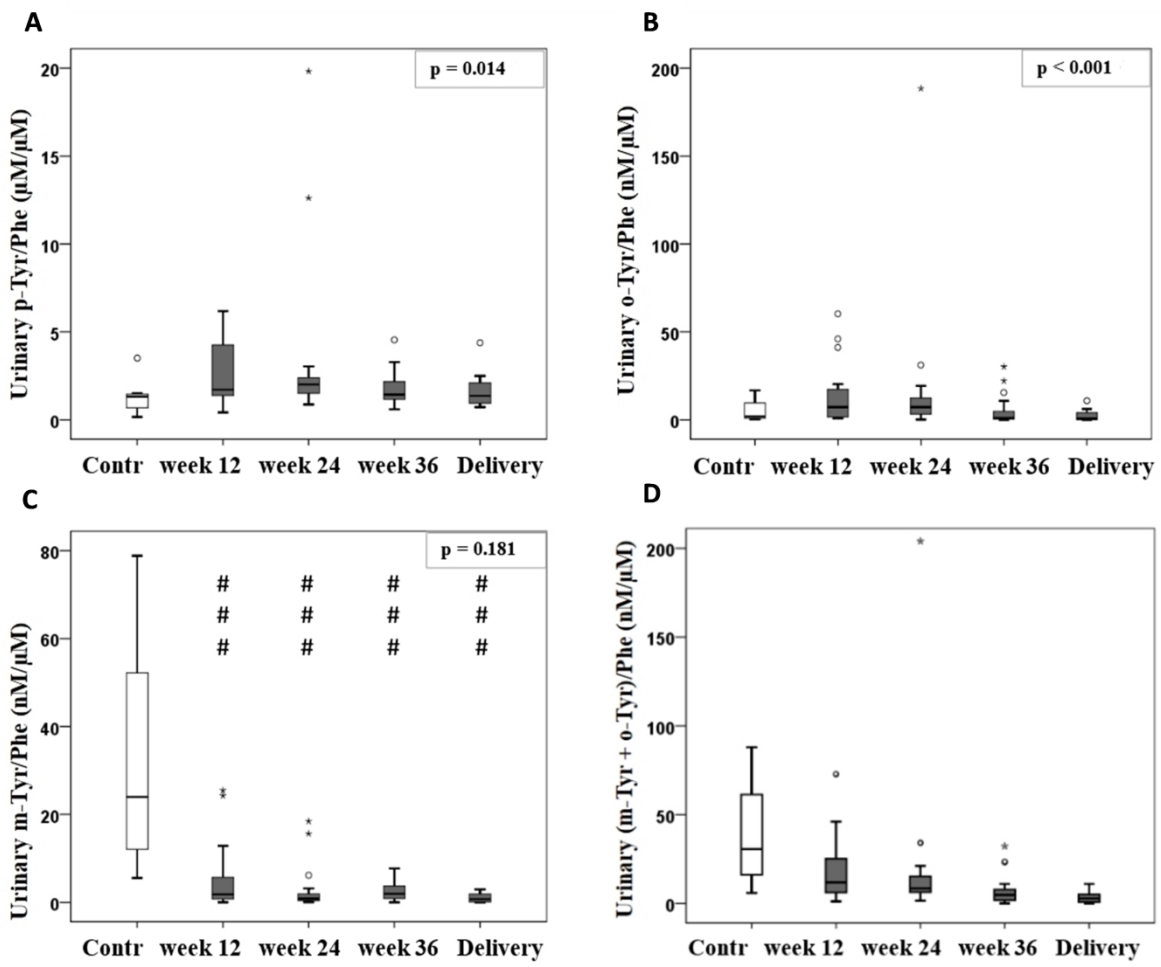

Figure 3: Urinary para-tyrosine/phenylalanine (A), ortho-tyrosine/phenylalanine (B), meta-tyrosine/phenylalanine (C) and (meta + ortho-tyrosine)/phenylalanine (D) ratios in non-pregnant controls and at 12, 24, 36 weeks of gestation and at delivery;
The p value in the inserted boxes of each panel represents the result of the Jonckheere-Terpstra test for linear trend
(#, p<0.05; ##, p<0.01; ###, p<0.001 vs. Contr)
p-Tyr, para-tyrosine; Phe, phenylalanine; o-Tyr, ortho-tyrosine; m-Tyr, meta-Tyrosine;

We found a significantly decreasing tendency of urinary o-Tyr/p-Tyr ratio during the gestation, while m-Tyr/p-Tyr ratios were neither significantly different compared to the control, nor a tendency could be detected.

Tendencies of serum haemoglobin, erythropoietin and serum erythropoietin/haemoglobin ratio during pregnancy

We observed that serum haemoglobin levels gradually decreased during pregnancy (Figure 4, Panel a), while serum EPO levels changed in an opposite pattern (Figure 4, Panel b), which lead to an increasing EPO/haemoglobin ratio (Figure 4, Panel c).

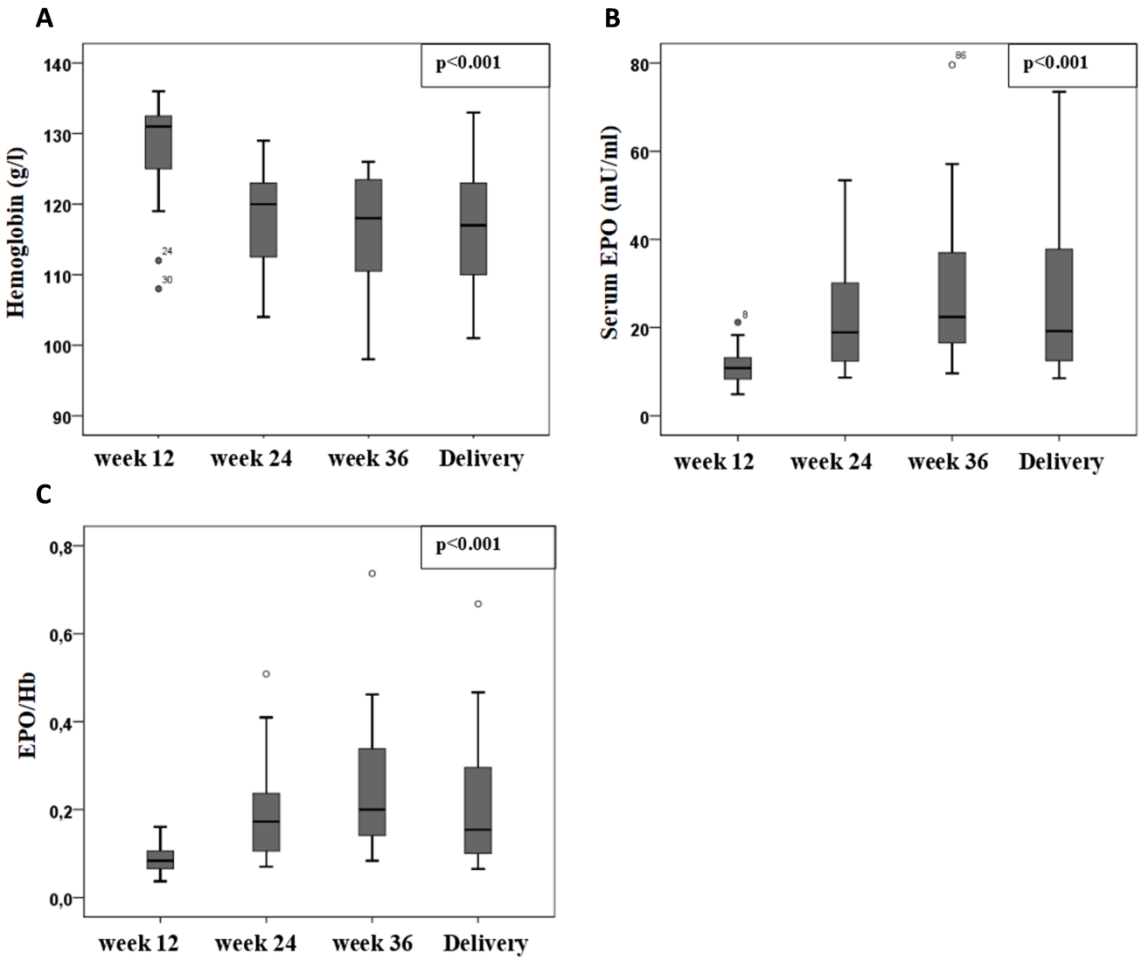

Figure 4: Levels of hemoglobin (A), erythropoietin (B), and the erythropoietin/hemoglobin ratio (C) at 12, 24, 36 weeks of gestation and at delivery
The p value in the inserted boxes of each panel represents the result of the Jonckheere-Terpstra test for linear trend
EPO, erythropoietin; Hb, hemoglobin

Association of parameters of erythropoiesis and serum- and urinary Phe, p-, m-, and o-Tyr levels
According to the data shown in Table 2, we found inverse correlation between the serum level of abnormal Tyr metabolites and the red blood cell count as well as haemoglobin and hematocrit and we also observed the same correlation with Phe. Anaemia worsened with increasing levels of these amino acid parameters. RDW-SD values, on the other hand, showed a consistent strong positive correlation with levels of urinary Tyr metabolites, especially o-Tyr and the o-Tyr/p-Tyr ratio, the higher the urinary excretion of abnormal Tyr isomers, the greater is the anisocytosis.

We observed, that EPO-levels were negatively correlated to red blood cell count, haemoglobin concentration, and hematocrit level, which presumably is a part of the normal feedback regulation of erythropoisesis. Furthermore, EPO levels showed positive correlation to increased serum p-Tyr and Phe levels. EPO resistance, calculated using the EPO/haemoglobin ratio, increased with elevated serum levels of Phe.

Table 2: Correlations of parameters of erythropoiesis and serum and urinary phenylalanine, para-tyrosine, meta-tyrosine, ortho-tyrosine levels in physiological pregnancies.

Clinical parameter	Amino acid parameters	r	p
RBC	Serum m-Tyr	-0.284	0.011
	Serum o-Tyr	-0.240	0.033
	Serum m-Tyr/p-Tyr	-0.243	0.031
	Serum o-Tyr/p-Tyr	-0.242	0.031
	Serum (m-Tyr+o-Tyr)/Phe	-0.240	0.033
	Serum (m-Tyr+o-Tyr)/p-Tyr	-0.328	0.003
Hb	Serum m-Tyr	-0.224	0.047
	Serum o-Tyr	-0.241	0.032
	Serum Phe	-0.281	0.012
	Serum (m-Tyr+o-Tyr)/p-Tyr	-0.281	0.012
Htc	Serum m-Tyr	-0.222	0.049
	Serum o-Tyr	-0.250	0.026
	Serum Phe	-0.250	0.026
	Serum (m-Tyr+o-Tyr)/p-Tyr	-0.276	0.014
RDW-SD	Urinary o-Tyr	0.283	0.019
	Urinary o-Tyr/Phe	0.251	0.037
	Urinary o-Tyr/p-Tyr	0.292	0.015
	Urinary (m-Tyr+o-Tyr)/p-Tyr	0.243	0.044
	Urinary o-Tyr/creatinine	0.247	0.042
EPO	RBC	-0.313	0.005
	Hb	-0.384	<0.001
	Htc	-0.294	0.009
	RDW-SD	0.251	0.030
	Serum p-Tyr	0.250	0.026
	Serum Phe	0.233	0.038
EPO/Hb	Serum Phe	0.224	0.048

Abbreviations: RBC, red blood cell count; Hb, haemoglobin; Htc, haematocrit; RDW-SD, red cell distribution width - standard deviation, EPO, erythropoietin; Phe, phenylalanine; m-Tyr, meta-tyrosine; o-Tyr, ortho-tyrosine; p-Tyr, para-tyrosine; r, correlation coefficient; n, number of cases; Method: Spearman.

Predictors of erythropoiesis

We examined the predictors of erythropoiesis using multivariate linear regression analysis. The results can be seen in Table 3. Body weight proved to be a positive predictor in all models in cases of red blood cells, haemoglobin and hematocrit, while transferrin was a negative predictor of haemoglobin and hematocrit.

Regarding the red blood cell count, the abnormal Tyr isomer serum m-Tyr, and in the model for hematocrit the serum m- + o-Tyr/p-Tyr ratio proved to be a negative predictor.

Urinary o-Tyr, o-Tyr/Phe, o-Tyr/p-Tyr and o-Tyr/creatinine ratios proved to be positive predictors of RDW-SD.

Finally we observed that transferrin levels were positive, while age was a negative predictor for EPO levels and EPO resistance.

Table 3: Predictors of erythropoiesis using linear regression analysis in physiological pregnancies.

Clinical parameter	Model	Predictor	β	p
RBC	1	Body weight Transferrin saturation	0.405 0.320	<0.001 0.004
		Serum m-Tyr	-0.217	0.046
	2	Body weight Transferrin saturation	0.393 0.359	0.001 0.001
	3	Body weight	0.393	0.001
		Transferrin saturation	0.359	0.001
	4	Body weight	0.393	0.001
		Transferrin saturation	0.359	0.001
	5	Body weight Transferrin saturation	0.393 0.359	0.001 0.001
Hb	6	Transferrin Body weight	-0.658 0.340	<0.001 <0.001
	7	Transferrin Body weight	-0.658 0.340	<0.001 <0.001
	8	Transferrin Body weight	-0.658 0.340	<0.001 <0.001
	9	Transferrin Body weight	-0.658 0.340	<0.001 <0.001
Htc	10	Transferrin Body weight	-0.535 0.447	<0.001 <0.001
	11	Transferrin Body weight	-0.535 0.447	<0.001 <0.001
	12	Transferrin Body weight	-0.535 0.447	<0.001 <0.001
	13	Transferrin Body weight	-0.480 0.426	<0.001 <0.001
		Serum (m-Tyr+o-Tyr)/p-Tyr	-0.189	0.045
RDW-SD	14	Urinary o-Tyr	0.316	0.016
	15	Urinary o-Tyr/Phe	0.362	0.005
	16	Urinary o-Tyr/p-Tyr	0.345	0.008
	17	Urinary o-Tyr/creatinine	0.389	0.003
EPO	18	Transferrin	0.611	<0.001
		Age	-0.405	<0.001
	19	Transferrin Age	0.611 -0.405	<0.001 <0.001
EPO/Hb	20	Transferrin Age	0.639 -0.387	<0.001 <0.001
	21	Transferrin Age	0.639 -0.387	<0.001 <0.001

Abbreviations: RBC, red blood cell count; Hb, haemoglobin; Htc, haematocrit; RDW-SD, red cell distribution width - standard deviation, EPO, erythropoietin; Phe, phenilalanine; m-Tyr, meta-tyrosine; o-Tyr, ortho-tyrosine; p-Tyr, para-tyrosine; β, correlation coefficient; n, number of cases.

Different rows represent different models. In all cases age, bodyweight, serum creatinine, serum iron, serum transferrin, transferrin saturation and serum ferritin and a certain amino acid parameter (listed below) were included into the models.

Amino acid parameters in different models: serum m-Tyr; 2: serum m-Tyr/p-Tyr; 3: serum o-Tyr/p-Tyr; 4: serum (m-Tyr+o-Tyr)/Phe; 5: serum (m-Tyr+o-Tyr)/p-Tyr; 6: serum m-Tyr; 7: serum o-Tyr; 8: serum (m-Tyr+o-Tyr)/Phe; 9: serum (m-Tyr+o-Tyr)/p-Tyr; 10: serum m-Tyr; 11: serum o-Tyr; 12: serum (m-Tyr+o-Tyr)/Phe; 13: serum (m-Tyr+o-Tyr)/p-Tyr; 14: urinary o-Tyr; 15: urinary o-Tyr/Phe; 16: urinary o-Tyr/p-Tyr; 17: urinary o-Tyr/creatinine; 18: serum p-Tyr; 19: serum Phe; 20: serum Phe; 21: serum (m-Tyr+o-Tyr)/Phe; Method: Multivariate linear regression

DISCUSSION

In our study we have shown the impact of altered levels of Tyr isomers on erythropoiesis in pregnancy. The levels of hydroxyl radical-derived Tyr isoforms along

with enzymatically produced p-Tyr levels showed remarkable changes during pregnancy. Serum p-Tyr levels were significantly lower at all timepoints in the gestational group, while Phe levels showed a rather moderate decrease, resulting in significantly lower p-Tyr/Phe ratios, furthermore, m-Tyr levels were also elevated and showed a steady increase during gestation. As a result of elevated m-Tyr and reduced p-Tyr as well as Phe levels, the (m-Tyr + o-Tyr)/Phe and (m-Tyr + o-Tyr)/p-Tyr ratios were also higher, in case of the abnormal Tyr isomers over Phe ratio, the increment was significant. The levels of hydroxyl radical-derived Tyr isoforms especially m-Tyr levels and the (m-Tyr + o-Tyr)/Phe and (m-Tyr + o-Tyr)/p-Tyr ratios proved to be inversely correlated to erythropoiesis parameters, which suggests that the shift between abnormal and physiological Tyr isomers influences erythropoiesis in a negative manner. In almost all tested models for RBC, Hb and Htc, the abnormal Tyr isoforms proved to be independent negative predictors. In most of the models bodyweight was found to be a positive predictor of erythropoiesis. A possible explanation for this could be a better overall nutritional state, considering oxidative stress was found to be enhanced as body weight increases.[16]

As previously discussed, despite increased EPO production we found decreasing Hb, RBC and Htc levels during pregnancy while MCV, MCH, MCHC levels remained normal throughout the gestation, showing, that pregnancy associated anaemia was not related to iron deficiency, rather EPO resistance. Our previous studies have shown that in case of increased pathological Tyr concentration and lowered physiological p-Tyr levels, the translational integration of abnormal Tyr instead of p-Tyr into the signaling proteins may cause alterations of the intracellular mechanisms following EPO-receptor activation, hence resulting in decreased EPO-efficiency, and insulin resistance.[15, 17]

As we have observed a near significant decrease in the urinary excretion of o-Tyr as well as a remarkable reduction in serum levels of this isomer, presumably there is a retention of the above in the tissues. We have observed that urinary o-Tyr showed positive correlation and also proved to be an independent positive predictor of RDW-SD values. Growing urinary excretion of o-Tyr results in elevated RDW values, which may be explained as a reinforcement of erythropoietic activity after o-Tyr has been released from the tissues, confirming that retention of ths abnormal isoform in the tissues interferes with red cell formation. Previous studies have shown a positive relation between RDW values and erythropoietic activity and the possibility that high serum EPO levels associated with elevated RDW values may indicate compromised bone marrow effects of EPO.[18]

CONCLUSION

According to our results we may infer that pregnancy associated anaemia is not due to iron deficiency alone, but it could be a consequence of EPO resistance as well, that may be in connection with the effect of the elevated elements of oxidative stress.

ABBREVIATIONS

erythropoiesis-stimulating agents (ESA).
erythropoietin (EPO)
estimated glomerular filtration rate (eGFR)
glomerular filtration rate (GFR)
High performance liquid chromatography (HPLC)
hydroxyl free radical (HO•)
mean corpuscular haemoglobin (MCH)
mean corpuscular haemoglobin concentration (MCHC)
Mean corpuscular volume (MCV)
meta- and ortho-tyrosine (m-Tyr, o-Tyr)
nicotinamide adenine dinucleotide phosphate (NADPH)
para-tyrosine (p-Tyr)
phenylalanine (Phe)
red cell distribution width (RDW)
red cell distribution width –standard deviation (RDW-SD)
renin-angiotensin-aldosterone system (RAAS)
trichloroacetic acid (TCA)
tyrosine (Tyr)
World Health Organization (WHO)

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DECLARATIONS

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that all have agreed on the submission and they have given necessary attention to ensure the integrity of the work. There are no conflicts of interest to be disclosed. This work is not under active consideration for publication, has not been accepted for publication, nor has it been published, in full or in part.

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Contribution to Authorship

I.T., I.W., G.A.M. and A.S. conceived and planned the study. I.T. enrolled the pregnant women, obtained the samples and carried out primary sample preparations. S.K. provided the control patient samples and performed laboratory procedures for HPLC and statistical analysis. A.M. contributed to parts of laboratory examinations. I. T. wrote the manuscript in consultation with I. W. and

A.S. All authors provided critical feedback and contributed to the interpretation of the results and the final version of the manuscript.

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