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Dynamics of serum levels and reference ranges of copeptin in the 3rd trimester of pregnancy in healthy pregnant women with uncomplicated pregnancy and delivery

Dynamika sérových hladin a referenční intervaly kopeptinu během III. trimestru těhotenství u zdravých těhotných žen s nekomplikovaným průběhem těhotenství a porodu

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Summary: **Objective:** Copeptin is a stable fragment of vasopressin. Copeptin levels have been found to reflect the degree of endothelial stress in various conditions, including acute coronary syndrome. Copeptin may be a biomarker for endothelial stress during pregnancy. However, there is still a lack of understanding of its dynamics and levels throughout pregnancy. This study aims to describe intra-individual and longitudinal changes in copeptin levels at 30th and 36th gestational weeks in healthy pregnant women with uncomplicated pregnancy and delivery and to establish specific reference ranges. **Methods:** A total of 125 pregnant women with uncomplicated pregnancy and delivery were included. These women were monitored throughout their pregnancy and gave birth at the Department of Obstetrics and Gynecology Olomouc University Hospital. The blood was taken at ~30 and ~36 gestational weeks. Serum copeptin levels were measured using a Kryptor Compact PLUS analyzer. For statistics, we used R software and the "referenceRanges" package. **Results:** It was found that serum levels of copeptin were significantly higher in the 36th week group than in the 30th week group ($P < 0.05$). Cook's distance was used to eliminate outliers. The 30th week median was 3.377 pmol/l, reference range = 1.343–7.829 pmol/l, and the 36 week was median 4.735 pmol/l and reference range = 2.06–13.2 pmol/l. In the 36th week reference range, the median was higher than in healthy, non-pregnant women ($P < 0.05$). Copeptin values can exceed 10 pmol/l, particularly after the 36th week. In the 3rd trimester, this value may indicate cardiovascular and endothelial overload. **Conclusion:** Copeptin levels were found to vary significantly depending on gestational week. The proposed reference ranges take into account the increased secretion of vasopressin in pregnancy. The existence of specific upper reference limits represents a potential advantage in detecting pregnant women prone to hypertensive disease in the 3rd trimester.

Key words: copeptin – vasopressin – preeclampsia – gestational hypertension – reference ranges – pregnancy

Introduction

Pregnancy can put a lot of strain on a woman's cardiovascular system due to volume overload, which can lead to hypertensive diseases. Preeclampsia (PE) is one of the most serious hypertensive diseases, causing significant maternal and perinatal morbidity and mortality worldwide [1]. Despite years of research, the exact cause of PE is still unknown. However, the main theory suggests that the placenta plays a significant role, with insufficient remodeling of the spiral arteries, placental insufficiency, and pathological activation of the vascular endothelium being major contributors [2,3]. Nevertheless, these findings are only present in a fraction of PE pregnancies [4]. Additionally, studies have shown that certain cardiovascular risk factors can increase the likelihood of developing preeclampsia [5,6].

Souhrn: **Cíl:** Kopeptin je stabilní fragment vazopresinu. Bylo zjištěno, že hladiny kopeptinu odrážejí stupeň endoteliálního stresu u různých stavů, včetně akutního koronárního syndromu. Kopeptin může být biomarkerem endoteliálního stresu během těhotenství, stále však chybí znalosti o jeho dynamice a hladinách v průběhu těhotenství. Cílem této studie je popsat intraindividuální a longitudinální změny hladin kopeptinu v 30. a 36. gestačním týdnu u zdravých těhotných žen s nekomplikovaným těhotenstvím a porodem a stanovit specifická referenční rozmezí. **Metody:** Do studie bylo zařazeno celkem 125 těhotných žen s nekomplikovaným těhotenstvím a porodem. Tyto ženy byly sledovány po celou dobu těhotenství a porodily na Porodnicko-gynekologické klinice FN Olomouc. Krev byla odebrána v 30. a 36. týdnu těhotenství. Hladiny kopeptinu v séru byly měřeny pomocí analyzátoru Kryptor Compact PLUS. Pro statistické zpracování byl použit software R a balíček „referenceRanges". **Výsledky:** Bylo zjištěno, že sérové hladiny kopeptinu byly významně vyšší v 36. týdnu než v 30. týdnu ($p < 0,05$). K vyloučení odlehlých hodnot byla použita Cookova vzdálenost. Pro 30. týden byl stanoven medián 3,377 pmol/l, referenční interval 1,343–7,829 pmol/l, pro ~36. týden byl stanoven medián 4,735 pmol/l a referenční interval 2,06–13,2 pmol/l. V 36. týdnu byl referenční interval i medián významně vyšší než u zdravých netěhotných žen ($p < 0,05$). Hodnoty kopeptinu překračují 10 pmol/l, zejména po 36. týdnu. Ve III. trimestru může tato hodnota indikovat zvýšenou kardiovaskulární a endoteliální zátěž. **Závěr:** Bylo zjištěno, že hladiny kopeptinu se významně liší v závislosti na gestačním týdnu. Navrhované referenční intervaly zohledňují zvýšenou sekreci vazopresinu v těhotenství. Existence specifických horních referenčních mezí představuje potenciální výhodu při identifikaci těhotných žen s rizikem vzniku hypertenzního onemocnění ve III. trimestru.

Klíčová slova: kopeptin – vazopresin – preeklampsie – gestační hypertenze – referenční interval – těhotenství

It seems that an insufficiently functioning cardiovascular system also plays a key role in the etiology of PE. Recent works confirm that there is a direct link between adaptation disorders and the risk of PE, especially late forms after the 34th gestational weeks (GW) [7]. Insufficient cardiac output leads to reduced uteroplacental perfusion and subsequently to stress-pathological activation of the endothelium of uteroplacental vessels.

This association has led to the investigation of existing and new biomarkers that are commonly used in the diagnosis of acute and chronic heart failure. Researchers have extensively studied the biomarkers mentioned in various studies [8–11].

One such promising biomarker is copeptin. It is a fragment of the prohormone for vasopressin, which is responsible for increasing vascular vasoconstriction and regulating water retention in the kidneys. This hormone is released in response to changes in plasma osmolality and reduced cardiac output. Copeptin, being a degradation product of vasopressin, is used as an indicator to determine serum vasopressin levels due to its greater stability [12].

The vasopressin-regulated stress-mediated hypothalamic-pituitary-adrenal axis plays a crucial role in the endocrine stress response [13]. Thus, it has been found that copeptin levels can indicate the degree of endothelial stress in various stress conditions, including acute coronary syndrome [14]. Several studies have suggested that increased levels of copeptin may play a role in the development of both early onset and late onset PE [15–18]. It is currently difficult to clinically use copeptin due to a lack of understanding of its levels during specific gestational weeks and how physiological changes during pregnancy can impact its concentration, particularly in the third trimester. In addition, it is important to note that the degree of standardization of the analytical determination of copeptin is very low, and therefore the values can differ significantly depending on the method used. It is also necessary to realize that the reference ranges used in studies were extrapolated from the non-pregnant population and provided by the manufacturer [9].

In this retrospective observational study, the objective was to determine the changes in serum copeptin levels during the third trimester in healthy pregnant women who had uncomplicated pregnancies and deliveries. The study aimed to assess the importance of these changes between different gestational weeks. Furthermore, we aim to propose reference ranges in monitored gestational weeks for KryptorCompact systems (BRAHMS).

Materials and methods

The study included 125 women, who were monitored throughout their pregnancy and gave birth at the Department of Obstetrics and Gynecology Olomouc University Hospital. Uterine artery Doppler flow was performed in women during the GW 30–32 and GW 36–37 of gestation from September 2019 to May 2021. Records of blood pressure, weight, body mass index (BMI), proteinuria, and estimated fetal weight were obtained during each visit. Proteinuria was determined using the Albumin-Creatinine Ratio (qACR) from a single urine sample according to the formula albumin (mg)/creatinine (mmol). BMI was calculated as weight (kg)/height^2 (m). Additionally, venous blood was drawn from all pregnant women during each visit. All pregnant women had data available from their obstetrics history, including previous pregnancies, anamnesis, birth, and newborn records.

Venous blood was collected in vacuum tubes with a clotting activator (Vacuette, Greiner). Samples were taken between 30. 8. 2019 and 6. 5. 2021 and were transported to the laboratory immediately after collection. Upon arrival, they were centrifuged, and the sepa-

rated serum was stored at –80 °C until analysis. Prior to analysis, samples were thawed only once and analyzed immediately in one run. The analysis for the study was done in June 2020 and September 2021. The sampling analysis was performed in the accredited laboratory of the Department of Clinical Biochemistry in the Olomouc University Hospital on the BRAHMS, KryptorCompact PLUS automatic analyzer. The ThermoScientific BRAHMS COPEPTIN US kits were used in this study as per the manufacturer's instructions. This is a homogeneous sandwich immunoassay based on the principle of TRACE technology (Time-Resolved Amplified Cryptate Emission) to determine serum copeptin levels. According to the B·R·A·H·M·S COPEPTIN US method, the total sample analysis time was 14 min and the sample volume required was 50 µl. The calibration range of this method spreads out from 0.9 to 500 pmol/l. It is noteworthy that none of the values were found to be outside this calibration range.

All participants in the study gave informed consent and the study was approved by the Ethical Review Committee of the Olomouc University Hospital (n. 35/22, March 7th, 2022).

Statistical analysis

The study utilized a non-parametric test to compare the copeptin values in pregnant women in the 30 and 36 GW. A Shapiro-Wilk normality test was conducted, revealing a P-value of 3.55×10^{-26}. The data didn't follow a normal distribution, which was confirmed later on. Thus, non-parametric methods were used to analyze the data. Linear regression using Cook's distance method was applied to eliminate statistical outliers in the study's data. The method used for estimating the reference ranges with respective 90% confidence intervals and median was the same as that used for calculating the 2.5 and 97.5 centiles. Values in individual weeks were compared using the Wilcoxon paired test. The statistical analysis for this study was conducted using the R program (R Core Team 2021) [19] and the "referenceRanges" package [20]. Graphs were constructed using the Microsoft Excel program.

Tab. 1. Characteristics of the cohort.
Tab. 1. Charakteristika souboru.

Parameters	Median/range/number
age (years)	33 (28–40)
BMI	22.9 (18.1–32.9)
race	caucasian
SBP at 30th–32nd gestational week	122 (95–139)
DBP at 30th–32nd gestational week	73 (51–90)
SBP at 36th–37th gestational week	124 (95–140)
DBP at 36th–37th gestational week	80 (60–90)
GA at first sampling	30+0 to 32+1
GA at second sampling	36+0 to 37+5
vaginal delivery	N = 125
GA at delivery (weeks + days)	36+1 to 41+4
birth weight (g)	3,490 (2,170–4,620)

BMI – body mass index, DBP – diastolic blood pressure, GA – gestational age, N – number, SBP – systolic blood pressure

Tab. 2. Values of copeptin at 30th and 36th gestational weeks.
Tab. 2. Hodnoty kopeptinu v 30. a 36. gestačním týdnu.

Biomarker	Gestational week	Median	Reference range	
			low limit	high limit
copeptin (pmol/l)	30	3.4	1.3	7.8
	36	4.7	2.1	13.2

Results

Characteristics of the cohort

The study included 125 pregnant women who met the inclusion criteria. Inclusion criteria were an uncomplicated singleton pregnancy, aged between 28 and 40 years, BMI ≤ 33 or less during the 1st trimester, no previous or gestational hypertension, no proteinuria, spontaneous or induced vaginal delivery after 36+0 weeks, and newborns weighing ≥ 10th percentile according to The National Newborn Standards (Intergrowth-21st) [21]. The characteristics of the cohort are given in Tab. 1. Five outliers were excluded from the values in ~30 GW and four outliers in ~36 GW. Gestational hypertension, preeclampsia, and fetal growth restriction were excluded based on blood pressure values (≤ 140/90 mmHg), proteinuria (qACR < 30 mg/mmol), normal Doppler parameters, and estimated fetal weight (EFW ≥ 10th percentile) according to The International Fetal Growth Standards (Intergrowth-21st) [22].

According to Tab. 2, the median's lowest and highest values of the 2.5 and 97.5 percentile for each gestational week are presented. It was found that the levels of copeptin increased depending on the gestational week. The median value given by the manufacturer for healthy non-pregnant women is 3.9 pmol/l, which is in line with the median at 30 GW. However, the median at 36 GW is significantly higher. Fig. 1 also illustrates the copeptin levels at 2 gestational periods and the shift of the median from

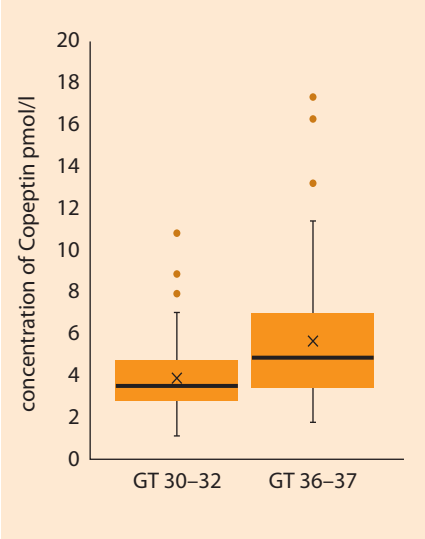

Fig. 1. Box plot of the copeptin concentration at 30th–32nd and 36th–37th gestational weeks.
Obr. 1. Krabicový graf znázorňující koncentraci kopeptinu v 30.–32. a 36.–37. gestačním týdnu.

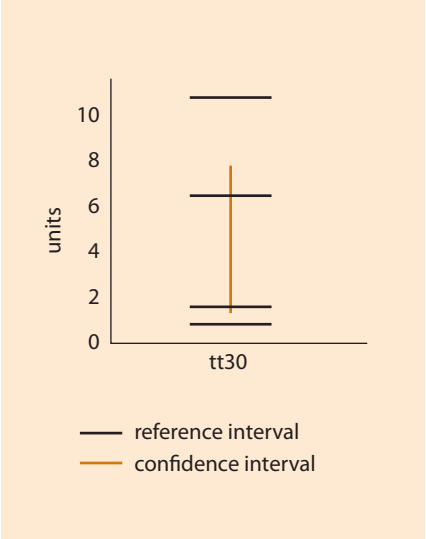

Fig. 2. Reference range for 30th gestational weeks.
Obr. 2. Referenční interval pro 30. gestační týden.

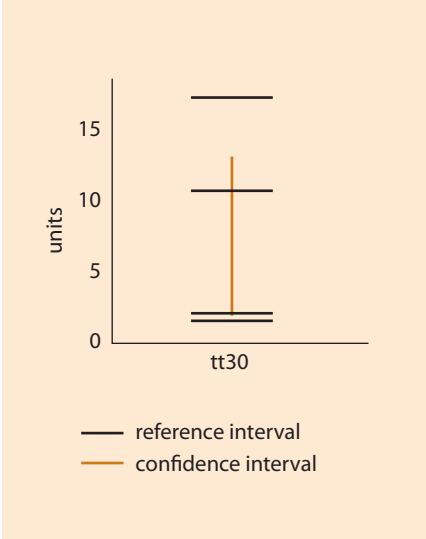

Fig. 3. Reference range for 36th gestational weeks.
Obr. 3. Referenční interval pro 36. gestační týden.

3.4 (30 GW) to 4.7 (36 GW). Furthermore, it was confirmed with a Wilcoxon paired test that there is a statistically significant difference between copeptin levels in pregnant women at 30 GW and 36 GW, with a P-value of 3.55×10^{-26}.

The proposed reference range (RR) for ~30 GW was 1.34–7.83 pmol/l, and for ~36 GW it was 2.06–13.2 pmol/l (Fig. 2, 3).

Fig. 1 and 3 clearly show that while the lower reference limit does not vary significantly, the median, and especially the upper reference limit, increases significantly with increasing gestational week.

Discussion

To date, only one study has been conducted to estimate reference ranges of copeptin in healthy pregnant women for the KryptorCompact plus device. This study, which included 62 pregnant women, found changes in copeptin values related to gestational age, which is consistent with our findings [23]. The manufacturer's reported median reference limit for non-pregnant women is 3.9 pmol/l. However, our results show that at the ~36 GW, the median value is significantly higher than the manufacturer's reported value. In contrast, 97.5% of the values in the study by Joose et al. remained below the manufacturer's reference limit.

The increasing production of vasopressin with gestational week is not surprising. The significant increase in cardiac output and body fluid volume in pregnancy is associated with arterial vasodilation and a decrease in arterial blood volume. The consequence is an increased glomerular filtration rate combined with sodium and water retention. Adaptation in pregnant women is manifested by increased vasopressin secretion to osmotic challenges. It is interesting to note that during the 2nd and 3rd trimesters of pregnancy, the placenta produces an increased amount of vasopressinase, which is responsible for the breakdown of vasopressin. However, this compensation is not sufficient to prevent the increase in plasma vasopressin during pregnancy, which can lead to vasoconstriction and an increase in blood pressure due to vasopressin's action via the V1 receptor [24,25].

The continuous increase in vasopressin during pregnancy has been confirmed by several studies, although most of these studies have focused on the use of copeptin as a novel biomarker of preeclampsia. Santillan et al. measured copeptin levels in the first, second, and third trimesters in a cohort of pregnant women with preeclampsia, a control group, and a group of non-pregnant women [15]. Significantly higher concentrations were found in the control group than in the group of non-pregnant women, starting in the 1st trimester. Tuten et al. also found in their study that copeptin concentrations in the control groups were associated with gestational age [17]. Marek et al. monitored copeptin levels by ELISA in 21 pregnant women who developed gestational hypertension and 37 pregnant women in the control group. The levels were determined in the 1st, 2nd and 3rd trimesters. The median in the control group for the 1st trimester was 51.5 pg/ml, for the 2nd trimester it was 59.1 pg/ml, and for the 3rd trimester it was 66 pg/ml. Despite the different methods of determination, a significant difference was again found between trimesters with an increase in concentration with gestational week [26].

Significant findings were that the upper reference ranges at ~36 GW were higher than the manufacturer's cut-off value of 10 pmol/l for the diagnosis of heart failure. It is suggested that the increasing stress on the cardiovascular system of t pregnant women during the 3rd trimester could be the cause of high vasopressin concentrations. The question is whether the increase in late pregnancy is due to osmotic or non-osmotic causes and whether the secretion is solely from the brain [27].

In line with the study by Joosen et al., we recommend the use of reference ranges specific to pregnancy, gestational age, and assay methodology for use in clinical trials, particularly to distinguish elevated vasopressin concentrations due to preeclampsia or gestational hypertension [23].

Conclusion

To conclude, there was a significant difference in copeptin levels depending on gestational week. However, the changes between GW were within the reference ranges for non-pregnant women without risk of heart failure. The proposed ranges take into account the increased vasopressin secretion in pregnancy. Specific upper reference limits have the potential benefit of identifying pregnant women at higher risk of hypertension in pregnancy. For use in clinical trials, particularly to distinguish elevated vasopressin concentrations due to preeclampsia or gestational hypertension, it is appropriate to use reference ranges specific to pregnancy and gestational age and to the assay methodology.

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