A Preliminary Study over Second Trimester Biochemical Markers and Their Clinical Utility

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Identification of early markers of pregnancy complications is a goal of materno-fetal medicine. The main purpose of the study was to see if changes in second trimester markers concentration are associated with IUGR and PIH complicated pregnancies. Was analyzed second trimester biochemical markers concentration in seven groups of pregnant women: with birth at term without pregnancy complications (BT) (Group A; n=1628), with SGA without PIH (Group B; n=39), with PIH without SGA (Group C; n=26), with PIH and SGA (Group D; n=6), with PIH or SGA (Group E; n=71), with PIH including SGA (Group F; n=32), and SGA including PIH (Group G; n=45). Second trimester hCG concentration is higher in BT group compared to group E (1.07±0.01 vs. 0.93±0.05, p=0.08) and group G (1.07±0.01 vs. 0.93±0.04, p=0.05), whereas uE3 concentration is lower in group A compared to group D (0.95±0.09 vs. 1.28±0.27; p=0.07), E (0.95±0.09 vs. 1.01±0.05; p=0.08) and group G (0.95±0.09 vs. 1.12±0.11; p=0.06). Our study showed that hCG value is higher while uE3 is lower in group A compared to group E and group G pregnant women.

Keywords: second trimester, biochemical markers, prediction, SGA, PIH

Second trimester biochemical markers screening combined with first or second trimester ultrasound measurements was used in the past as the first choice for aneuploidy screening. New protocols developed in the last 20 years switch the aneuploidy screening earlier in the first trimester of pregnancy [1]. However, the second trimester screening still remains a valid choice for pregnant women who missed the first trimester screening [2]. For these pregnant women there arises the question if second trimester biochemical markers could be useful to evaluate the risk of other pregnancy-associated disorders such as small for gestational age (SGA), pregnancy induced hypertensive disorders (PIH), preeclampsia (PE) or preterm birth (PB).

The classic second trimester screening consists in the measurement of three biochemical markers (alfa-fetoprotein, hCG, and free Estriol) in sera of pregnant women between 15 and 22 weeks of pregnancy (wp).

Alpha-fetoprotein (AFP) is a protein encoded by a gene located on the q arm of chromosome 4 (4q25) [3-5]. During pregnancy AFP is produced by the yolk sac and the liver and decrease in Down syndrome fetuses and elevate in fetal disorders (neural tube defect, omphalocele) or maternal disorders (tumors, hepatoma, etc.) [6-8].

Human chorionic gonadotropin (hCG) is a glycoprotein hormone normally secreted by trophoblastic cells of the placenta during pregnancy [9]. hCG is a heterodimeric glycoprotein with an α (alpha) subunit identical to that of luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and a β (beta) subunit that is unique to hCG. hCG levels changes are related to certain fetal chromosomal abnormalities and birth defects. Elevated hCG values are related to germ cell and trophoblastic tumors.

Estriol, figure 1, is produced during pregnancy by the placenta from 16-hydroxydehydroepiandrosterone sulfate (16-OH DHEAS) an androgen steroid made in the fetal liver and adrenal glands [10].

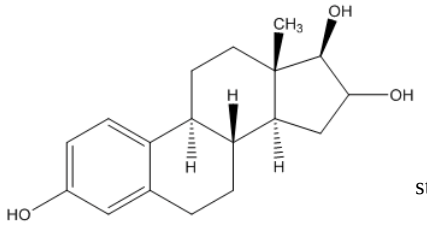

Fig. 1. Chemical structure of estriol

The human placenta produces pregnenolone and progesterone from circulating cholesterol. Pregnenolone is converted in the fetal adrenal gland into dehydro-epiandrosterone (DHEA), then subsequently sulfonated to dehydroepiandrosterone sulfate (DHEAS). DHEAS is converted to 16-OH DHEAS in the fetal liver. The placenta converts 16-OH DHEAS to estriol, and is the predominant site of estriol synthesis. Free estriol values reflect both the

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fetal wellbeing and the placenta activity. Frees estiol values are decreased in chromosomal or congenital anomalies such as Down syndrome or Edward's syndrome [11].

Intrauterine growth restriction (IUGR) refers to a condition in which a fetus is unable to achieve its genetically-determined potential size [12]. IUGR is determined by a complex pathology caused by maternal factors, placental diseases or hormonal disturbances [13,14]. IUGR is associated with a high risk of neonatal complication and stillbirth [15].

Hypertension disease in pregnancy (PIH) is defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, based on the average of at least 2 measurements, taken at least 15 min apart, using the same arm [16]. Hypertensive disorders of pregnancy affect around 5-8% of pregnancies and are associated with increased risks of perinatal morbidity and mortality [17].

Since mechanisms that lead to IUGR and PIH are activated long time before clinical signs occur, we analyze herein if changes in second trimester markers concentration is associated with IUGR and PIH complicated pregnancies.

Experimental part

Patients and sera

Sera were collected between 15 and 22 weeks of pregnancy from singleton pregnant women (n=1699). Each pregnant woman was followed until birth. Data about pregnancy outcome, were obtained by retrospectively analyzing the medical files. The pregnant women were classified according to the outcome in pregnant women with birth at term without pregnancy complications (BT) (Group A; n=1628), with SGA without PIH (Group B; n=39), with PIH without SGA (Group C; n=26), with PIH and SGA (Group D; n=6), with PIH or SGA (Group E; n=71), with PIH including SGA (Group F; n=32), respectively SGA including PIH (Group G; n=45).

Measurement of second trimester biochemical markers

Second trimester biochemical markers (AFP, hCG and E3) were measured with the help of chemiluminescence method, using an ImmuliteOne Machine (DPC, Diagnostic Products Corporation, Los Angeles, USA) and commercially available kits (Siemens Healthcare Diagnostics Products Ltd., Llanberis, Gwynedd, LL55 4EL, UK). Values were expressed in corrected multiple of medians, calculated according to PRISCA software, Version 4 (Typolog Software, Tomesch, Germany). Data from pregnant women and biochemical markers were stored using ASTRAIA software, the materno-fetal module (Astraia GmbH, Munich, Germany).

Ethical issues

The research meets the conditions of the ethical guidelines and legal requirements and was approved by the Committee for Ethics Research of the University of Medicine and Pharmacy Timisoara. Informed consent was obtained from every patient.

Statistical analysis

Data are expressed in median+/- Standard error of mean (SEM). The statistical significance of the differences between two groups was assessed using Mann-Whitney U tests.

Results and discussions

Identification of early markers of pregnancy complications is a goal of materno-fetal medicine [18]. Since SGA and PIH are serious complications of pregnancy the detection of new markers associated with them could pave the way for an earlier diagnosis, development of new screening methods and could help to explain the pathological mechanisms involved in the etiology of these disorders. Although the new guidelines recommend that the screening of PIH and SGA should be performed in the first trimester of pregnancy [1], since some pregnant women still come for their first antenatal visit only in the second trimester, the question whether second trimester markers could predict the risk of PIH and SGA makes practical sense.

Alpha-fetoprotein (AFP) second trimester serum concentration in pregnant women with BT, SGA and PIH

No difference in AFP concentration was found between pregnant women with BT (Group A) and pregnancies with SGA without PIH (Group B) (0.92+/-0.03 *vs.* 0.84+/-0.05), PIH without SGA (Group C) (0.92+/-0.03 *vs.* 0.91+/-0.04), PIH and SGA (Group D) (0.92+/-0.03 *vs.* 0.71+/-0.23), PIH or SGA (Group E) (0.92+/-0.03 *vs.* 0.87+/-0.04), PIH including SGA (Group F) (0.92+/-0.03 *vs.* 0.90+/-0.05), SGA including PIH (Group G) (0.92+/-0.03 *vs.* 0.84+/-0.05) (table 1).

Since PIH and in certain cases SGA are caused by disturbances in the placentation process it was expected that concentration of biochemical markers that are synthetized by placenta could be useful in early diagnosing of these pregnancy complications.

Human chorionic gonadotropin hormone (hCG) second trimester serum concentration in pregnancies with BT, SGA, and PIH

No difference in hCG concentration was found between pregnant women with BT (Group A) and pregnancies with SGA without PIH (Group B) (1.07+/-0.01 *vs.* 0.93+/-0.05),

		AFP	b-hCG	uE3
A	Pregnant women with birth at term and without pregnancy complications	0.92±0.03	1.07±0.01	0.95±0.09
B	Pregnant women with SGA without PIH	0.84±0.05	0.93±0.05	1.09±0.11
C	Pregnant women with PIH without SGA	0.91±0.04	1.05±0.11	0.94±0.12
D	Pregnant women with PIH and with SGA	0.71±0.23	0.91±0.09	1.28±0.27
E	Pregnant women with PIH or SGA	0.87±0.04	0.93±0.05	1.04±0.08
F	Pregnant women with PIH including SGA	0.90±0.05	0.93±0.09	1.01±0.05
G	Pregnant women with SGA including PIH	0.84±0.05	0.93±0.04	1.12±0.11
A vs. B		ns, p=0.54	ns, p=0.11	ns, p=0.18
A vs. C		ns, p=0.34	ns, p=0.69	ns, p=0.69
A vs. D		ns, p=0.28	ns, p=0.21	p=0.07
A vs. E		ns, p=0.18	p=0.08	p=0.08
A vs. F		ns, p=0.18	ns, p=0.37	ns, p=0.26
A vs. G		ns, p=0.34	p=0.05	p=0.06

Table 1
SECOND TRIMESTER BIOCHEMICAL MARKERS CONCENTRATION IN PREGNANT WOMEN WITH BIRTH AT TERM WITHOUT COMPLICATIONS (BT), PREGNANCY INDUCED HYPERTENSION (PIH), AND SMALL FOR GESTATIONAL AGE (SGA)

PIH without SGA (Group C) (1.07+/-0.01 vs 1.05+/-0.11), SGA and PIH (Group D) (1.07+/-0.01 *vs.* 0.91+/-0.09), PIH including SGA (Group F) (1.07+/-0.01 *vs.* 0.93+/-0.09). A difference was found between second trimester hCG concentration in pregnant women with birth at term and without complications (Group A) and PIH or SGA (Group E) (1.07+/-0.01 *vs.* 0.93+/-0.05, p=0.08), SGA including PIH (Group G) (1.07+/-0.01 *vs.* 0.93+/-0.04, p=0.05) (table 1).

Our results showed that the second trimester AFP concentrations are not different among the seven groups of pregnant women while hCG and uE3 concentrations showed some differences in certain categories of pregnant women.

Free estriol (uE3) second trimester serum concentration in pregnancies with SGA, PIH, and without complication

No difference in uE3 concentration was found between pregnant women with BT (Group A) and pregnancies with SGA without PIH (Group B (0.95+/-0.09 *vs.* 1.09+/-0.11), PIH without SGA (Group C) (0.95+/-0.09 *vs.* 0.94+/-0.12), PIH including SGA (Group F) (0.95+/-0.09 *vs.* 1.01+/-0.05).

A difference was found between second trimester uE3 concentration in pregnant women with birth at term and without pregnancy complication (Group A) and PIH and SGA (Group D) (0.95+/-0.09 *vs.* 1.28+/-0.27; p=0.07), PIH or SGA (Group E) (0.95+/-0.09 *vs.* 1.04+/-0.08; p=0.08) and respectively SGA including PIH (Group G) (0.95+/-0.09 *vs.* 1.12+/-0.11;p=0.06) pregnant women (Table 1).

Our results showed that second trimester hCG concentration is higher in pregnant women without pregnancy complications and birth at term compared to pregnant women with PIH or SGA and SGA including PIH. Also second trimester uE3 concentration is lower in pregnant women without pregnancy complications and birth at term compared to pregnant women with PIH and SGA, with PIH or SGA, respectively SGA including PIH. Interestingly, previous research showed that second trimester AFP and hCG concentrations are elevated in cases with preeclampsia compared to pregnant women with birth at term without complication while uE3 showed no difference [19,20] . Other studies evaluated the combined predictive value uterine Doppler velocity and second trimester AFP or hCG concentration and found that IUGR cases are associated with elevated AFP and hCG values [21]. In another study a better predictive value was achieved by combining second trimester markers with the presence of the uterine artery notch [22]. A meta-analysis performed by Hui *et al* showed that no identifiable combination of serum markers performs well as a screening test for preeclampsia or SGA [23].

The influence of some biological markers in early pregnancy of patients with birth at term and preterm birth was studied in another study [24].

Conclusions

In conclusion, even if individual studies find that second trimester biochemical markers could predict SGA or PIH, one of the performed meta-analyses does not confirm these results. Our study showed that second trimester hCG concentration is higher while uE3 concentration is lower in pregnant women without pregnancy complications and birth at term compared to pregnant women with PIH or SGA, and SGA including PIH.

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