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THE INFLUENCE OF FETAL SEX AND MOTHER'S SMOKING AND PARITY ON AFP AND FREE β -HCG CONCENTRATIONS IN AMNIOTIC FLUID OF UNAFFECTED SECOND TRIMESTER PREGNANCIES

UTJECAJ SPOLA FETUSA I PUŠENJA TE RODNOSTI MAJKE NA AFP I SLOBODNI β -HCG U PLODOVOJ VODI UREDNIH TRUDNOĆA DRUGOG TROMJESEČJA

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Original paper

Key words: amniotic fluid; alpha-fetoprotein; free β -hCG; fetal sex; parity; smoking

SUMMARY. Objective. The aim is to investigate the influence of mother's smoking and parity and fetal sex on AFP and free β -hCG in amniotic fluid and to examine the correlation between maternal serum and amniotic fluid marker concentrations. **Methods.** The study was performed on 233 second-trimester amniotic fluid samples. In 75 women, blood sample was taken immediately before amniocentesis too. All pregnancies were singleton with normal fetal karyotype and outcome. Concentrations of AFP and free β -hCG were determined by fluoroimmunoassay and converted to MoM, according to medians for unaffected pregnancies of the corresponding gestational age. **Results.** In smoking women, amniotic fluid free β -hCG was significantly lower than in non-smoking ones ($p=0.033$), though AFP was not significantly different in regard to smoking habits ($p=0.113$). Significantly higher amniotic fluid free β -hCG ($p<0.001$) and lower AFP ($p=0.015$) were found in female, in comparison to fetal male gender. No significant change according to parity, neither for free β -hCG, nor for AFP was observed ($p=0.094$ and $p=0.376$, respectively). Significant correlation of AFP to free β -hCG was found between serum and amniotic fluid samples ($r=0.61$, $p<0.001$ and $r=0.35$, $p<0.002$, respectively). **Conclusions.** Our results confirmed the influence of fetal sex on amniotic fluid AFP and free β -hCG and negative influence of smoking on free β -hCG as well as on maternal serum AFP and free β -hCG concentrations. More data should be available to determine the impact of parity on examined amniotic fluid markers.

Izvorni rad

Cljučne riječi: plodova voda, alfa-fetoprotein, slobodni β -hCG, spol fetusa, paritet, pušenje

SAŽETAK. Cilj istraživanja. (1) Ispitati utjecaj pušenja i rodnosti (pariteta) majke i spola fetusa na razinu alfa-fetoproteina (AFP) i slobodne β -podjedinice humanog korionskog gonadotropina (slobodni β -hCG) u plodovoj vodi urednih trudnoća. (2) Ispitati korelacije između serumskih vrijednosti AFP i slobodnog β -hCG i vrijednosti spomenutih biljega u plodovoj vodi. **Ispitanice i metode.** Ispitivanje je provedeno na 233 uzorka plodove vode koji su dobiveni amniocentezom u drugom tromjesečju trudnoće. U 75 slučajeva uzet je i uzorak krvi neposredno prije amniocenteze. Sve su trudnoće bile jednoplodne, urednog tijeka i ishoda, a kariotip ploda bio je uredan. Koncentracije AFP i slobodnog β -hCG određivane su fluoroimunometrijskom metodom i izražene u *multiples of median* (MoM) za odgovarajući tjedan trudnoće. **Rezultati.** U plodovoj vodi trudnica pušačica utvrđena je statistički značajno niža razina slobodnog β -hCG nego u nepušačica ($p=0,033$). Utjecaj pušenja na razinu AFP u plodovoj vodi nije utvrđen ($p=0,113$). Nasuprot tomu, spol fetusa je značajno utjecao na razinu slobodnog β -hCG i AFP u plodovoj vodi: razina slobodnog β -hCG je bila statistički značajno viša, a AFP značajno niža ako je fetus bio ženskog spola ($p<0,001$ za slobodni β -hCG; $p=0,015$ za AFP). Vrijednosti istraživanih biljega u plodovoj vodi nisu se mijenjale u odnosu na paritet ($p>0,05$ za oba biljega). Statistički značajna korelacija utvrđena je za koncentracije AFP ($r=0,61$; $p<0,001$) i slobodnog β -hCG ($r=0,35$; $p<0,002$) između seruma i plodove vode. **Zaključak.** Rezultati ovog istraživanja potvrđuju utjecaj spola fetusa na vrijednosti slobodnog β -hCG i AFP u plodovoj vodi kao i negativan utjecaj pušenja na razinu slobodnog β -hCG. Promjene su istovjetne onima u serumu. Utjecaj pariteta na ispitivane biljega u plodovoj vodi nije potvrđen, moguće zbog nedovoljnog broja uzoraka.

Introduction

Despite the great interest of numerous authors in co-variables influencing serum biochemical markers for prenatal screening of Down syndrome (DS) and neural tube defects (NTD), there are still insufficient data concerning its distribution in amniotic fluid. Some reports

tried to clarify if the changes of second-trimester pattern of human chorionic gonadotropin (hCG) or its free sub-

* **Acknowledgements:** This research was supported by grant (No. 0062017/2002–2006) of Ministry for Science, Education and Sports, Zagreb, Republic of Croatia.

units (free β -hCG, free β -hCG) as well as alpha-fetoprotein (AFP), mirrored on its levels in amniotic fluid.^{1–3} The aim of the present study is to investigate possible co-influence of smoking habits, parity and fetal sex on AFP and free β -hCG levels in amniotic fluid, as has been earlier well documented for maternal serum.^{4,5} Particular interest has been paid to find out if there is a correlation of AFP and free β -hCG levels between maternal serum and amniotic fluid in second-trimester unaffected pregnancies.

Materials and Methods

A total of 233 samples of amniotic fluid, from pregnant women who underwent amniocentesis between 15 and 20 weeks' gestation, were collected through the period from April 2004 to February 2005. In 68% of cases (n=158) the procedure was undertaken on the basis of advanced maternal age, in 18% (n=42) on the basis of elevated risk in biochemical and/or ultrasonographic screening, and in 14% of women (n=33) because of the risks from genetic or family disease history. Out of total, 75 of studied women (32%) gave the informed consent for taking blood sample, immediately before amniocentesis. All were singleton pregnancies, none of them was conceived with methods of assisted reproduction and there were no cases of insulin-dependent diabetes mellitus. The necessary patient's data (maternal age and weight, smoking habits and previous gravidity/parity) were taken at the time of amniocentesis in a standard manner, exactly as for biochemical screening. All pregnant women were white and of the same ethnic origin. Gestational age was confirmed or established by ultrasonographic fetal biometry prior to the invasive procedure. For this study, we selected only pregnancies with normal fetal karyotype and those in which progress and normal outcome was confirmed from obstetric and delivery room databases.

Amniotic fluid samples were centrifuged at 2000 rpm for 10 min; amniocytes were then cultured for karyotyping. The supernatant was frozen at -20°C till analysis, together with the corresponding serum sample. Bio-

chemical markers, alpha-fetoprotein and free β -hCG, were determined by fluorometric immunoassay DELFIA (Perkin Elmer Life and analytical sciences, Wallac Oy, Turku, Finland). Samples of amniotic fluid for measuring AFP and free β -hCG concentrations were diluted, using zero-standard point, up to 1:100 and 1:5, respectively. Concentrations of each marker were expressed in multiples of the median (MoM) for unaffected pregnancies of the corresponding gestational age. Medians of serum markers were derived from a routine serum-screening program and were adjusted for maternal weight. Regressed medians for gestation between 15th and 21st weeks were used for calculation of MoM values of amniotic fluid markers.

Statistical analysis utilized descriptive statistics for population parameters, χ^2 -test for testing proportions and one-way ANOVA for estimating differences between continuous variables. The MoMs of MSAFP and free β -hCG were transformed in corresponding \log_{10} MoMs in order to obtain a normally distributed set of values, which allowed the application of parametric statistical tests. Kolmogorov-Smirnov tests of goodness-to-fit to a Gaussian distribution were demonstrated for both markers ($p>0,20$). Statistical significance was determined at p level of 0.05. Pearson's coefficient was used for estimating correlation of markers between serum and amniotic fluid.

Results

The mean maternal age of studied pregnant women (n=233) was 33.8 ± 5.5 (mean \pm SD) and the gestational age was 16.5 ± 5.5 . *Table 1* shows the characteristics of the study population, according to smoking status, parity and fetal sex. We found significantly lower MoM values of free β -hCG in amniotic fluid (AF free β -hCG MoM) of smoking pregnant women, compared to non-smoking ones ($p=0.033$) (*Figure 1*). In distinction to that, amniotic fluid AFP MoM (AF AFP MoM) values between smokers and non-smokers were not significantly different ($p=0.113$) (*Figure 1*).

There were no significant differences in proportions of women with female and male fetuses, neither in rela-

Table 1. Characteristics of the study population according to smoking status, parity and fetal gender
Tablica 1. Karakteristike istraživane populacije u odnosu na pušački status, paritet i spol fetusa

	No. of cases – Broj trudnoća n (%)	Free β -hCG MoM		AFP MoM	
		Median	Mean $\log_{10}\pm$ SD	Median	Mean $\log_{10}\pm$ SD
Non-smokers – Nepušačice	195 (83.7)	1.03	-0.0242 ± 0.2203	0.89	-0.0257 ± 0.1932
Smokers – Pušačice	38 (16.3)	0.76	-0.1102 ± 0.2518	1.01	0.0283 ± 0.1809
Previous births – Raniji porodi					
0	78 (33.5)	1.06	-0.0054 ± 0.2448	0.95	0.0000 ± 0.2166
1	108 (46.3)	1.02	-0.0328 ± 0.2043	0.92	-0.0082 ± 0.1890
2	37 (15.9)	0.79	-0.0997 ± 0.2516	0.86	-0.0608 ± 0.1408
3	10 (4.3)	0.67	-0.1261 ± 0.1915	0.85	-0.0797 ± 0.1672
Fetal gender – Spol fetusa					
female – ženski	120 (51.5)	1.09	0.0260 ± 0.2075	0.89	-0.0465 ± 0.1856
male – muški	113 (48.5)	0.79	-0.1064 ± 0.2286	0.96	0.0145 ± 0.1943

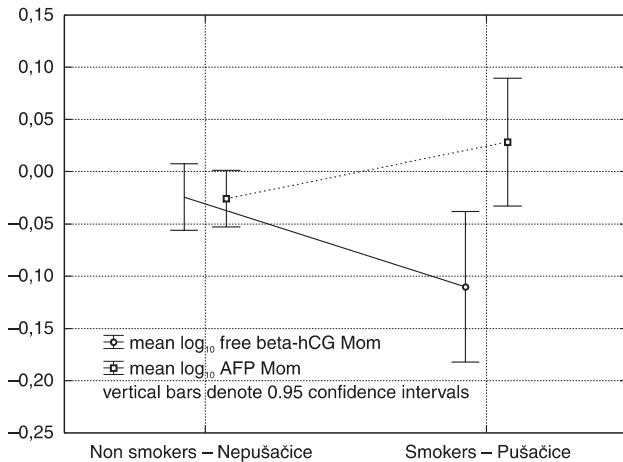


Figure 1. Mean \log_{10} MoMs of amniotic fluid free β -hCG and AFP according to smoking habits

Slika 1. Srednja vrijednost \log_{10} MoM slobodnog β -hCG i AFP u plodovoj vodi ovisno o pušačkom statusu trudnice

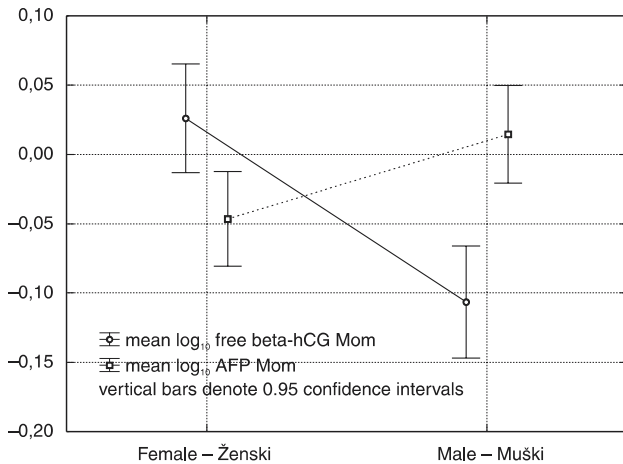


Figure 2. Mean \log_{10} MoMs of amniotic fluid free β -hCG and AFP according to fetal gender

Slika 2. Srednja vrijednost \log_{10} MoM slobodnog β -hCG i AFP u plodovoj vodi ovisno o spolu fetusa

tion to the number of previous births between non-smokers and smokers ($p > 0.05$). We revealed statistically significant difference between MoMs of both, AF free β -hCG and AF AFP in relation to fetal sex ($p < 0.001$ and $p = 0.015$, respectively) (Figure 2). AF free β -hCG MoMs were significantly higher in cases of pregnancies with female than in those with male fetuses. On the other hand, AF AFP MoM values in female pregnancies were significantly lower than in male pregnancies. No significant differences in proportions of smokers and non-smokers and in proportions of women according to parity, between male and female pregnancies, were found ($p > 0.05$). We found no significant changes with regard to parity, neither of AF free β -hCG MoM, nor of AF AFP MoM values ($p = 0.094$ and $p = 0.376$, respectively).

In 75 paired samples the correlation between serum and amniotic fluid AFP and free β -hCG MoM values

was analyzed. Statistically significant positive correlation was found for \log_{10} free β -hCG MoM values ($r = 0.61$, $p < 0.001$). On the other side, positive, although weak correlation was found for \log_{10} AFP MoM values ($r = 0.35$, $p = 0.002$). Moreover, significant positive correlation between serum and amniotic fluid free β -hCG MoM values was determined in smokers and non-smokers ($r = 0.71$, $p = 0.003$ and $r = 0.58$, $p < 0.0001$, respectively). We also found significant correlation of free β -hCG MoM values between serum and amniotic fluid, in both female and male pregnancies ($r = 0.54$, $p = 0.0003$ and $r = 0.74$, $p < 0.0001$, respectively).

Discussion

The aim of the present study was to investigate the influence of cigarette consumption, parity and fetal sex on amniotic fluid concentrations of free β -hCG and AFP, as well as to investigate the correlation of biochemical markers between matched serum and amniotic fluid. As we mentioned in our previous report⁶, women submitted to the program of prenatal care in Croatia constituted an almost homogenous population, regarding race and ethnic origin, so they represented a suitable model for research of the influence of co-factors on biochemical markers.

It has been known that AFP concentrations in the amniotic fluid of normal pregnancies are 10^2 – 10^3 times higher than in maternal serum at the same gestation and that levels decrease with increasing gestational age, while concentrations in maternal blood increase with gestational age.² On the other side, the concentrations of free β -hCG are 5–10 times higher in amniotic fluid than those in maternal serum and decrease with gestational age in both fluid compartments.^{1,2}

Our results confirmed good and significant positive correlation for free β -hCG MoM values between maternal serum and amniotic fluid during second trimester of pregnancy. Previously, similar observations were reported on total hCG in the second and third trimester.^{3,7} On the other hand, we found weak, although significant correlation for AFP MoMs between maternal circulation and amniotic fluid compartment. Still, with sample size presented in this study, we detected the correlation significant, with power level of 80% ($\alpha = 0.05$). Some earlier studies have shown no significant correlation between AFP concentrations in maternal serum and amniotic fluid.^{8,9} The authors supposed that simple diffusion might not be the only mechanism for the transfer of AFP from amniotic fluid to maternal serum. As had been postulated, the main contribution of AFP in amniotic fluid, after 14 weeks' gestation, is reached via fetal kidneys and urine. In normal pregnancies, two mechanisms are responsible for fetal-to-maternal transfer: transplacental, driven by hydrostatic gradient across the placental villous surface and other, with the passage through the basal plate, in which AFP gains entrance to maternal vessels that traverse decidua basalis and basal

plate.^{10,11} It has also been elucidated that placental tissues produce hCG and its subunits throughout pregnancy. Namely, syncytiotrophoblast, which is the major component of chorionic villi, secretes hCG and its subunits directly into maternal blood. On the other side, amniotic fluid that resides the compartments surrounding the trophoblastic tissue also contains a certain amount of hCG and, even significant amount of its α - and β -subunit.¹ Our results demonstrate good and significant correlation for free β -hCG MoM values between maternal circulation and amniotic fluid compartment, no matter of fetal sex, and also independently of maternal smoking habits.

The present study shows significantly depressed free β -hCG MoM values in amniotic fluid of smoking pregnant women in relation to non-smoking subjects. We reported the same effect on second-trimester maternal sera in our earlier investigation⁴, which was a continuation of some previous studies concerning negative influence of smoking on serum concentration of total hCG^{12,13} and free β -hCG.^{14,15} On the other hand, the effect of smoking on maternal serum AFP concentrations has been less evident.^{14,15,16} Our earlier results obtained on maternal sera,⁴ as well as results on amniotic fluid presented here, are on the same trail. Slightly increased AFP MoM values in amniotic fluid of our smoking women, compared to non-smokers, are not significantly different. Verspyck et al.¹⁷ reported similar observation.

Although the impact of fetal sex on maternal serum concentrations of AFP and free β -hCG has been well documented in literature,^{5,18–22} the attitude towards the influence on biochemical markers in amniotic fluid is still erratic. In the present study, significantly higher amniotic fluid free β -hCG MoM and lower AFP MoM values were found in pregnancies with female in comparison to those with male gender. Spencer et al.² found consistently higher free β -hCG MoM values in all gestational weeks, in amniotic fluid of unaffected pregnancies with female fetuses, but they could not prove any significant differences in AF AFP MoM values in relation to fetal sex. Drugan et al.²³ found similar AF AFP MoM values in singleton pregnancies regardless to male or female fetus. Some other studies confirmed that amniotic fluid of male fetuses more often contained elevated AFP MoM values, compared to those in females.^{17,24} In spite of uneven conclusions in the literature, our results are supporting the hypothesis that gender specific regulatory mechanisms are responsible for different production, secretion and feto-maternal transfer of placental and fetal proteins, which is evident in both fluid compartments.

In our previous investigation on maternal serum markers we were unable to document a clear trend of changes for MS AFP MoM values, in relation to parity.⁴ This was also in agreement with the results of Spencer²⁵ and Wald.²⁶ Recently, Lei et al.²⁷ in an extensive population study, described the increased occurrence of absolute and relative proportion of low maternal serum AFP lev-

els in line with the increased parity. In the present study, the results have not shown any coherent association between MoM values of AF AFP and the number of previous births. Also Verspyck et al.¹⁷ did not observe significant difference in mean AF AFP MoM, in relation to parity. In our previous report, we confirmed significantly higher free β -hCG MoM in sera of primigravidas, while increasing parity negatively affected the average free β -hCG MoM values.⁴ On the other side, free β -hCG MoM values in amniotic fluid samples presented in this study have shown decreasing trend with parity, although the significance of the direction hasn't been proved. These data cannot be compared with the literature, for the lack of reports on free β -hCG in amniotic fluid in relation to previous gravidity and parity.

In conclusion, our results confirm the influence of fetal sex on amniotic fluid AFP and free β -hCG MoM values and, also, the influence of cigarette smoking on amniotic fluid free β -hCG MoMs. No significant differences, for neither of studied amniotic fluid biochemical markers, according to parity were observed. Significant, good positive correlation was demonstrated for free β -hCG MoM values between maternal serum and amniotic fluid. Regarding the lack of agreement among these and previously reported results, further investigation will be required to determine the possible influence of co-variables on amniotic fluid biochemical markers.

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Paper received: 7. 10. 2006; *accepted:* 30. 11. 2006.

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