# Does Active Smoking Influence the Second Trimester Biochemical Markers Concentrations?

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*Cigarette smoke contains over 7000 different substances some of them exerting harmful effects on embryo and pregnant woman. Nowadays 15 % of adult people and around 10-15% of pregnant women smoke. Previous studies showed that cigarette smoke compounds could exert pharmacodinamic effects and influence some of the second trimester biochemical markers concentration. Therefore there is a need to adjust the reference values of second trimester markers depending of the smoker status. The aim of our study was to analyse which of the markers are influenced by smoking and whether the software used to calculate the risk for aneuploidies is able to counterbalance this influence. Alpha-fetoprotein (AFP), chorionic gonadotropin hormone (hCG) and free estriol (uE3) values were measured in second trimester sera of 1242 pregnant women: 1089 non-smokers and 153 smokers. Only hCG second trimester values were influenced by smoking whereas AFP and uE3 values were not. The correction of medians according to the smoking status was able to counterbalance this effect.*

*Key words: second trimester biochemical markers, aneuploidies, smoking, correction*

There are many strategies to screen for fetal aneuploidies [1]. Over the years technological achievements in the field of laboratory chemistry and ultrasound medicine made it possible to create strategies for aneuploidies screening in the first trimester of pregnancy [2]. Nowadays the *gold standard* promoted by the group of scientists around the Fetal Medicine Foundation is a combined screening that includes measurement of ultrasound markers (crown-rump-length, nuchal translucency thickness, nasal bone, ductus venosus Doppler and tricuspid Doppler) and biochemical markers (free-β-hCG and PAPP-A) [2]. Although it is considered the most effective method in aneuploidies screening other strategies are implemented around the world. These are still in use because of different reasons: (1) some pregnant women come too late for the first trimester screening; (2) some physicians consider that the delay of screening to the early second trimester offers more ultrasonographic information about the fetus; (3) not all centers own the infrastructure or the qualification to run a first trimester screening program.

Before implementing a combined first trimester strategy in our center, for many years we ran an integrated protocol in which we measured first trimester ultrasound markers (crown-rump-length and nuchal translucency) and early second trimester biochemical markers [alpha-fetoprotein (AFP), human-chorionic gonadotropin (hCG), and free Estriol (uE3)] [3]. According to the algorithm, the measured value of each ultrasonographic/biochemical marker is related to a median value which represents the expected median value that is found in the population of pregnant women who carry healthy fetuses. The deviation of a measured value from the median is expressed in multiple of medians (MoM) and is used to calculate a relative risk for aneuploidy. Because it was proved that other parameters (mode of conceiving, smoking, ethnicity, diabetes, etc) interfere with the level of biochemical markers, it is mandatory to correct the MoM according to mentioned parameters [4-7]. Thus a corrected MoM (MoMc) is calculated for each biochemical marker considering interfering parameters.

Active smoking is a widespread habit that affects around 1 billion people worldwide [8,9]. Cigarette smoke contains a huge amount of chemicals out of which nicotine is the main dependence-inducing one. Other substances are hydrogen cyanide, formaldehyde, lead, arsenic, ammonia, benzene, nitrosamines, carbon monoxide, and polycyclic aromatic hydrocarbons [10]. Some of these substances may harm embryo during pregnancy [11], influence homeostasis [12], cause cancer or other (heart, bladder, kidney, etc.) diseases [13]. Because a significant percentage of our patients are active smokers, in our study we aim to analyze the influence of tobacco smoke upon the value of second trimester biochemical markers and

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the ability of the aneuploidy risk evaluation software to counterbalance this effect.

## Experimental part

*Patients and sera*

An integrated aneuploidy screening program that includes first trimester nuchal translucency measurement and second trimester biochemical markers (AFP, hCG and uE3) was applied to 1242 pregnant women (singleton pregnancies, spontaneously conceived pregnancies, without diabetes). Nuchal translucency thickness and crown-rump length were measured between 11+4 and 13+6 weeks of pregnancy (wp) and second trimester biochemical markers between 15 wp and 22+6 wp. Pregnant women were interrogated about the date of the last menstrual period, mode of conceiving, smoking behavior, diabetes and weight at the time of biochemical screening. In all pregnancies the gestational age was determined based on first trimester crown-rump length measurement. Only pregnant women with spontaneously conceived pregnancy, without diabetes and singleton pregnancy were included in our study. The pregnant women were classified according to their smoking status into: 1089 non-smokers and 153 smokers.

*Measurement of second trimester biochemical markers*

Second trimester biochemical markers (AFP, hCG and uE3) were measured by the 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 multiple of medians (MoM) and corrected multiple of medians (MoMc), calculated according to the PRISCA software, Version 4 (Typolog Software, Tomesch, Germany). Data from pregnant women and biochemical markers were stored using the ASTRAIA software, the maternal-fetal module (Astraia GmbH, Munich, Germany) [14,15].

*First trimester ultrasound markers measurement*

Crown-rump length was measured according the fetal medicine foundation (FMF) guidelines.

*Ethical issues*

The research meets the conditions of the ethical guidelines and legal requirements and was approved by the Committee 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). GraphPad InStat software, San Diego, California, USA and SPSS, IBM Inc. were used for statistical analysis. Mann-Whitney sum of ranks test was used to compare series of values.

## Results and discussions

Integrated first and second trimester aneuploidies screening implies measurement of crown-rump length (CRL) and nuchal translucency (NT) thickness in the first trimester and of biochemical markers in the second trimester of pregnancy. Measurement of CRL in the first trimester allows an exact determination of gestational age. Since the concentration of each biochemical marker is compared with the value of a gestational age specific median, an exact determination of gestational age is mandatory [16]. In order to exclude errors caused by an inappropriate determination of gestational age only pregnancies with a gestational age which was determined based on CRL measurement were included in our study. Moreover, to avoid comparison of data from singleton and multiple pregnancies, only singleton pregnancies were included in our statistics.

The algorithm of individual risk evaluation cumulates the risk determined by the age of the pregnant women and by the gestational age and the risk calculated by relating the values of ultrasound markers (NT, nasal bone, etc) and biochemical markers (AFP, hCG, and uE3) to the gestational age specific median. Thus, the multiple of median (MoM) is a conventional measurement unit that reflects how far the value of a measured parameter (NT or biochemical markers) is from the median value expected for the same gestational age. Because studies showed that some other factors (behavioral or diseases) influence the median value, it is obvious that it is necessary to correct the MoM according to the presence of these factors [4-7]. So, the corrected multiple of median (MoMc) represents the MoM value adjusted for the presence of interfering factors (mode of conception, presence of diabetes, smoker status, ethnicity, etc). Because we analyze the effect of smoking on the biochemical markers value and the ability of the software to correct the influence of smoking, in our study we enrolled only Caucasian pregnant women, with singleton pregnancy, who conceived spontaneously and without diabetes.

*Demographic features of pregnant women: smokers vs non-smokers*

The mean age of pregnant women (non-smokers vs smokers) at the time of screening was 28.62 years vs. 27.81 years, the gestational age was 116.55 days vs. 117.5 days, and the weight was 61.50 kg vs. 62.00 kg. Only gestational age of non-smokers was lower than that of smokers (table 1).

*Influence of smoking on second trimester biochemical markers values (AFP, hCG, uE3)*

Second trimester biochemical markers values were measured in sera of non-smoking and smoking pregnant women: AFP represented in table 2.

*Ability of software to correct the influence of smoking on second trimester biochemical markers values*

Corrected multiple of median (MoMc) values were calculated for each of the second trimester biochemical markers in sera of non-smoking and smoking pregnant women. The corrected values showed a good capacity of the software to correct the influence of smoking (table 3).

Aneuploidy risk evaluation [17-19] is alongside the evaluation of risk of other pregnancy complications such

| | non-smokers | smokers | significance |
|---|---|---|---|
| Age (years) | 28.62±0.14 | 27.81±0.38 | 0.146 (ns) |
| Gestational age (days) | 116.00±0.22 | 118.00±0.74 | 0.006 |
| Weight (kg) | 61.50±0.366 | 62.00±0.96 | 0.67 (ns) |
| Number of pregnant women | 1089 | 153 | |

*Data are expressed in median ± SEM*

**Table 1**
DEMOGRAPHIC FEATURES OF PREGNANT WOMEN INCLUDED IN THE STUDY

**Table 2**
STATISTICAL EVALUATION OF SECOND TRIMESTER BIOCHEMICAL MARKERS VALUE IN NON-SMOKER (n=1089) VERSUS SMOKER PREGNANT WOMEN (n=153).

| | non-smokers | smokers | p-value |
|---|---|---|---|
| AFP | 1.04±0.01 | 1.04±0.03 | 0.59 (NS) |
| hCG | 0.90±0.01 | 0.69±0.04 | <0.0001 |
| uE3 | 0.99±0.01 | 0.95±0.02 | 0.65 (NS) |

*Values are expresses in multiple of medians (MoM)*

**Table 3**
STATISTICAL EVALUATION OF SECOND TRIMESTER BIOCHEMICAL MARKERS VALUES CORRECTED FOR SMOKING IN NON-SMOKER (n=1089) VERSUS SMOKER PREGNANT WOMEN (n=153).

| | non-smokers | smokers | p-value |
|---|---|---|---|
| AFP | 0.99±0.01 | 0.95±0.02 | 0.65 (NS) |
| hCG | 0.85±0.01 | 0.87±0.05 | 0.36 (NS) |
| uE3 | 0.98±0.01 | 0.96±0.03 | 0.34 (NS) |

*Values are expresses in corrected multiple of medians (MoMc)]*

as preterm birth [20], maternal-fetal infection transmission [21,22] or thyroidopathies [23] a standard of care in the majority of developed countries. Since ultrasound proved to be a safe method, it became, together with serological investigation, a useful method in the evaluation of early pregnancies [24]. Aneuploidies screening tests are evaluated according to their performance to select from the entire population those cases with the highest risk. This goal could be reached only if the risk calculation algorithm takes into consideration all parameters on the basis of which the risk is calculated. That is why in our study we analyzed the influence of smoking on second trimester biochemical markers concentration and the ability of our software to correct this influence.

Our results showed that the value of hCG concentration is lower in smoker compared to non-smoker pregnant women. If we did not have a correction for this effect, smoking would determine a false reduction of calculated risk of aneuploidy in pregnant women who smoke. The software reverses the influence of smoking. We don't know the exact mechanism through which chemicals from smoke reduce the hCG concentration. Further studies which analyze the correlation between the number of smoked cigarettes and the magnitude of influence on hCG concentration will be able to refine the algorithm of aneuploidy risk calculation.

## Conclusions

Our research confirms that smoking influences the second trimester hCG serum concentration and this could influence the results of aneuploidies screening. Adjustment of hCG values according to smoking status corrects this influence.

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