

CLINICAL IMPLICATIONS OF THE INDOLERGIC SYSTEM AND OXIDATIVE STRESS IN PHYSIOLOGICAL GESTATIONAL HOMEOSTASIS

ALINA CRENGUȚA NICOLAE^{1#}, CRISTINA MANUELA DRĂGOI^{1#}, IULIANA CEAUȘU^{2*#},
CRISTIAN POALELUNGI^{2#}, DOMINIC ILIESCU^{3#}, ANDREEA LETIȚIA ARSENE^{1#}

¹Department of Biochemistry, Faculty of Pharmacy, University of Medicine and Pharmacy "Carol Davila", 6, Traian Vuia str, sector 2, Bucharest, Romania

²Faculty of Medicine, University of Medicine and Pharmacy "Carol Davila", Department of Obstetrics and Gynecology, Dr. I. Cantacuzino Hospital, 5-7 Ion Movila, Bucharest, Romania

³Faculty of Medicine, University of Medicine and Pharmacy Craiova, Romania

*corresponding author: iulianaceausu2004@yahoo.com

#All authors had equal contribution to this research.

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Abstract

The physiological processes of reproduction and a normal intrauterine development ensure the proper functioning of the future adult human body. Physiological pregnancy is characterized by complex changes of the maternal body, which are based on specific biochemical mechanisms.

In the present study, there were investigated the normal serum variations of two major neurohormones: melatonin and serotonin and also, the maternal oxidative status in physiological pregnancy, along with the evolution of the gestational age. The main objective of this research was to establish a scientific basis, statistically substantiated, for the future use of these parameters as predictive biochemical markers for some diseases that may compromise the normal development of physiological pregnancy.

The experimental results were mathematically processed and we established for the first time in literature, predictive mathematical equations for the normal variation of the investigated biochemical markers, during physiological pregnancy.

Rezumat

Procesele fiziologice de reproducere și dezvoltare intrauterină normală asigură buna funcționare a viitorului organism uman adult. Sarcina fiziologică se caracterizează prin modificări complexe ale organismului matern, ce au la bază mecanisme biochimice particulare.

În studiul prezent, au fost determinate variațiile serice normale ale melatoninei și serotoninei, precum și statusul oxidativ matern, în sarcina fiziologică, odată cu evoluția vârstei gestaționale.

Obiectivul principal al acestei cercetări a fost stabilirea unor baze științifice, argumentate statistic, pentru utilizarea în viitor a acestor parametri ca markeri biochimici predictivi ai afecțiunilor ce pot influența negativ evoluția normală a unei sarcini fiziologice.

Rezultatele experimentale au fost prelucrate informatic și am stabilit pentru prima dată în literatură, ecuații matematice predictive pentru variația normală a markerilor biochimici investigați, pe durata unei sarcini fiziologice.

Keywords: melatonin, serotonin, oxidative status, pregnancy

Introduction

A particular aspect of the molecular logic of the living, perhaps the least elucidated in terms of science, is the process of reproduction.

It is known that nowadays the uniqueness of the human architecture is due to the interconnection of the regulatory systems (neural, endocrine, immune, genetic), but the molecular mechanisms that are the basis of this network are still incomplete elucidated [7].

In this context, the physiological processes of reproduction and normal intrauterine development ensure the proper functioning of the future adult human body.

Physiological pregnancy is characterized by complex changes of the maternal body (hormonal, neuronal, cardiovascular, immunological, adaptive changes), which are based on specific biochemical mechanisms. These molecular mechanisms ensure a normal development and status for both mother and foetus, throughout the gestation period.

From this point of view, any biochemical imbalance can be the basis of many gestational diseases: hyperglycaemia, preeclampsia, depression and even spontaneously miscarriage [6, 12, 18].

The involvement of the indolergic system, represented by the interrelation of the melatonergic

system with the serotonin system, in maintaining the normal gestational homeostasis is a highly topical issue in the international scientific research. The headquarters of the melatonergic system is the epiphysis (the pineal gland). Although the pineal gland was associated by C. I. Parhon, since 1938, with the growth disorders and sexual maturation (e.g. precocious puberty), the molecular mechanisms that form the basis of this disease are not yet fully known.

With the discovery of melatonin as the main hormone secreted by the epiphysis, it was issued the hypothesis of its involvement in the reproductive processes. In the specialty literature there are studies demonstrating the regulatory role of melatonin on the hypothalamic-pituitary-gonadal axis [5].

Thus, the administration of melatonin in laboratory animals produced a significant decrease of the serum concentration of testosterone, of the luteinizing hormone, of the follicle-stimulating hormone, of prolactin, and an increase in the concentration of progesterone.

Some researchers sustain that melatonin, beside its circadian pulsatility, also suffers a biosynthesis variation dependent on the ovarian cycle, and an increase of the concentration of melatonin in the luteal phase [13].

The latest researches in this area demonstrated the involvement of melatonin in the sleep-awake rhythm imbalance and in the thymic tonus in pregnant women and its antioxidant protecting effect on the placenta and the foetus.

As a result, nowadays is being investigated the possibility of using the pineal hormone as a biochemical marker for the early diagnosis of some gestational diseases as preeclampsia, gestational diabetes, thromboembolism.

The second major component of the indolergic system, which regulates the gestational homeostasis, is the serotonergic system through its main representative, serotonin (5HT). Serotonin is currently considered a neuroendocrine informational transducer with a neuromediator role and as local hormone, acting both centrally and peripherally.

Recently it was stated that the level of the circulating serotonin in pregnant women majorly influences the normal development of the fetal brain, the serotonergic neurons of the foetus being identified since the 5th week of the pregnancy [1, 2]. Thus, an increased serum concentration of serotonin in the pregnant woman serum was positively correlated, statistically significant, with the incidence of the autism development in child [4]. Currently, the maternal serotonin imbalance is considered a major risk factor for the emergence and development of the autism in children [11, 16].

Another very important process for the development of normal pregnancy is the serotonin interaction with its specific receptors (5HT_{1D} and 5HT_{2B}) from pancreatic β cells, which highlights the fundamental modulator role of serotonin on the emergence and development of gestational diabetes.

All these researches that have demonstrated the major role of serotonin in maintaining the gestational homeostasis and the normal development of the foetus, are currently supported by Alexandre Bonnin and Pat Levitt from the Department of Neurobiology at the Keck School of Medicine, University of Southern California, United States of America, which first demonstrated, by *ex vivo* experimental studies on placental tissue, that the placenta itself biosynthesizes and secretes serotonin [3].

Based on these assumptions published in the scientific literature, serotonin may be considered a potential fundamental biochemical marker for the diagnosis of some gestational diseases [15].

Another characteristic biochemical marker for the maintenance and evolution of a normal pregnancy is the oxidative maternal status. Recent research studies showed major imbalances of the prooxidant-antioxidant systems of the maternal body, which could lead to morphological and functional damage to the foetus.

Several research studies showed the positive correlation of the oxidative maternal status with the incidence of some gestational disease (especially gestational diabetes and preeclampsia). Therefore, the oxidative status of the mother may be a biochemical predictive marker for the occurrence and development of some pathologies associated with pregnancy [9, 17, 19].

Based on these data from the literature, we decided to investigate the normal serum variations of melatonin and serotonin and also the maternal oxidative status in physiological pregnancy, along with the evolution of the gestational age.

The main objective of this research was to establish a scientific basis, statistically substantiated, for the future use of these parameters as predictive biochemical markers for some diseases that may compromise the normal development of physiological pregnancy.

Materials and Methods

Study design

In this clinical research there were included a total of 83 patients, Caucasian, aged 18-40 years, who presented at the Department of Obstetrics and Gynecology of the "Ioan Cantacuzino" Hospital from Bucharest, between March 2010-September

2011. There were selected only the patients who presented between 7-10 a.m.

The protocol has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Before enrolling in the study, all subjects gave the volunteered and signed consent to participate in the study.

In this clinical research there were included patients who met the following corresponding criteria: good general condition (absence of any pathology), absence of any pharmacotherapy, absence of any family history of genetic diseases. The patients were selected according to the gestational age and there were formed the following study groups: control, not-pregnant (10 patients), pregnant women with the gestational age 12 weeks (23 patients), pregnant women with the gestational age 22 weeks (26 patients), pregnant women with the gestational age 32 weeks (24 patients). The clinical research was approved by the Ethics Committee of "I. Cantacuzino" Hospital.

Blood samples

The blood samples were collected *a jeun*, between 7-10 a.m. by venepuncture from the antecubital vein in sterile vacutainers without anticoagulant. Within 3 hours after collection, the blood was centrifuged at 3000 rpm for 10 minutes to separate the serum. The serum was immediately separated, brought in Eppendorf tubes and stored at -20°C , until the determinations were performed. The serum was used in order to assess melatonin and serotonin concentrations and also the oxidative stress level, expressed as reactive oxygen species (ROS).

Reagents

Sulfuric acid, sodium permanganate, trichloroacetic acid, sodium hypochlorite, luminol, sodium hypochlorite, trichloroacetic acid, of analytical grade were provided by SIGMA, USA.

Methods

Serum melatonin concentration was determined by a chemiluminescent method. The indole nucleus of melatonin is oxidized by potassium permanganate in an acid medium. During the reaction, it is obtained the singlet oxygen intermediate form, species with a high chemical energy, extremely unstable and highly reactive, due to the opposite spin electron. The transformation of the singlet oxygen to triplet oxygen, the most stable chemical species and the lowest energy oxygen, is carried with the emission of a light quanta (a luminescent signal). The intensity of the luminescence signal is directly proportional to the concentration of melatonin in the sample [8, 14, 20]. Serum ROS (reactive oxygen species) assay was based on a

method which involved a chemiluminescent reaction, namely the oxidation of luminol (5- amino -2,3 - dihydro -1,4 - ftalazindione) by sodium hypochlorite. During the reaction, it is obtained diazachinone, that in the presence of reactive oxygen species (ROS) is transformed into a aminophthalic molecule (a dicarboxylate dianion), the dianion being in an excited state. Aminophthalate, when passing from the excited to a basal state, emits a photon which is registered as a chemiluminescent signal that lasts 2 seconds. The intensity of the luminescence signal emitted is directly proportional to the concentration of reactive oxygen species participating in the reaction [10, 18, 20]. Serum serotonin assay was performed by an ELISA method. The ELISA kit was provided by Gen Way Biotech Inc.

Apparatus

The chemiluminometric studies were conducted using a Perkin Elmer chemiluminometer LS 50B model equipped with a thermostatic external system and a magnetic stirring system.

The ELISA assay was performed using the Automated EIA and Chemistry Analyser Chemwell 2010 system (Awareness Technology INC, USA).

Results and Discussion

The clinical research was conducted on 83 patients distributed into several groups, according to the gestational age (Figure 1).

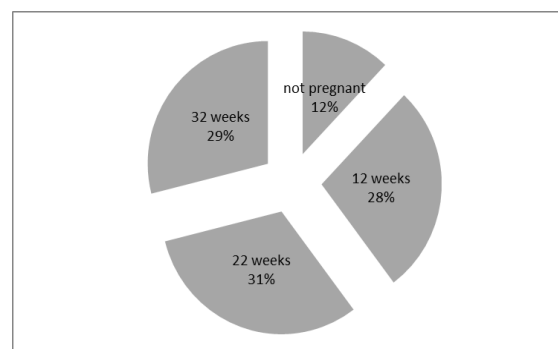


Figure 1.

Patients distribution on study groups, according to the gestational age

For each patient from the analysed groups there were determined the serum concentrations of melatonin, serotonin and the level of oxygen reactive species (as marker of the oxidative status). Figure 2 presents some representative graphics registered in order to determine the serum concentration of melatonin by the chemiluminometric method.

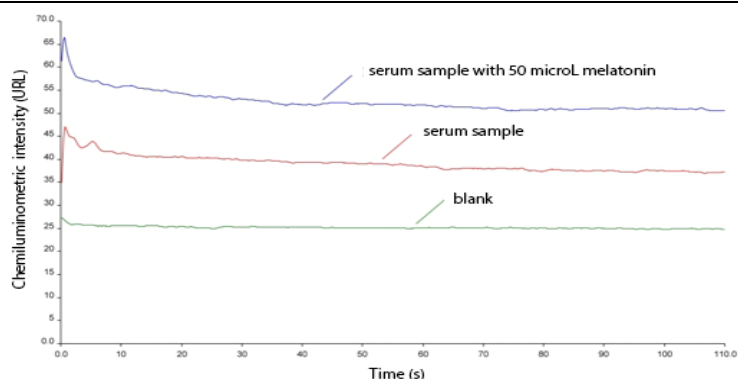


Figure 2.

Representative graphics registered during the chemiluminometric determination of the serum concentration of melatonin

Figure 3 presents some representative graphics registered in order to determine the serum concentration of reactive oxygen species, by the chemiluminometric method.

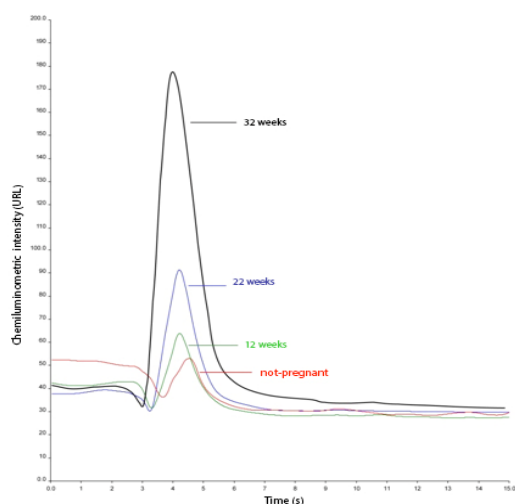


Figure 3.

Representative graphics registered during the determination of the serum concentration of reactive oxygen species

Figures 4, 5 and 6 show the graphical interpretation of serum concentrations of melatonin, serotonin and reactive oxygen species for the patients included in the study. The serum concentration of melatonin in the case of control (not pregnant women) was of 2.53 ± 0.99 pg/mL. During the course of normal, physiological pregnancy, the serum concentration of the pineal hormone increased so that at 12 weeks of pregnancy there were recorded the values of 2.72 ± 1.03 pg/mL, and at 22 weeks 5.49 ± 2.92 pg/mL. The highest plasma level of melatonin was obtained when it was assessed the group of patients in the third quarter of pregnancy (32 weeks), determining a mean value of 13.25 ± 2.83 pg melatonin/mL. This increase is statistically significant compared to the group of patients with a gestational age of 12 weeks and of 22 weeks ($p < 0.01$).

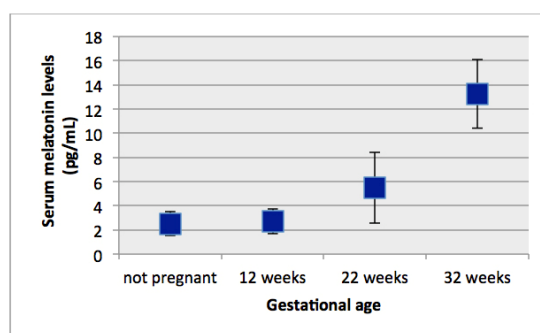


Figure 4.

Dynamics of the serum concentration of melatonin, depending on the gestational age of the patients

The serum concentration of serotonin in the studied pregnant patients registered an increase directly proportional to the gestational age. At the beginning of the pregnancy (12 weeks - 162.09 ± 76.55 ng/mL) the serum level of serotonin registers a decrease compared to the level registered for the non-pregnant patients (178.92 ± 67.14 ng/mL), so that, during the normal evolution of the pregnancy there were registered increasing concentrations of serotonin: 22 weeks - 240.53 ± 112.59 ng/mL and 32 weeks - 430.34 ± 117.21 ng/mL, statistically significant compared to the group of patients with a gestational age of 12 weeks and 22 weeks, respectively ($p < 0.01$).

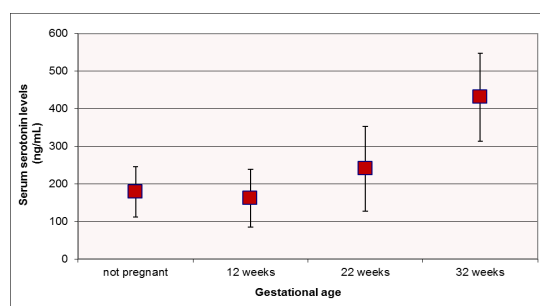


Figure 5.

Dynamics of the serum concentration of serotonin, depending on the gestational age of the patients

A similar evolution was observed when assessing the serum concentration of reactive oxygen species. According to the gestational age of the patients, it was registered a proportional increase in the level of oxidative stress. At 12 weeks of pregnancy (63.91 ± 17.13 url), reactive oxygen species level was comparable to that determined for non-pregnant patients (50.87 ± 22.73 url), and during the physiological evolution of pregnancy, it was observed a significant increase of their level: 22 weeks - 97.3 ± 36.60 url, 32 weeks - 199.07 ± 51.47 url, compared with the group of patients with a gestational age of 12 weeks ($p < 0.001$), respectively 22 weeks ($p < 0.01$).

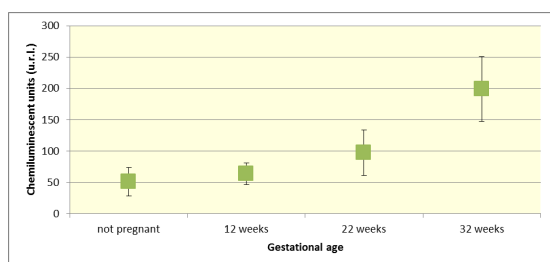


Figure 6.

Dynamics of the serum concentration of reactive oxygen species, depending on the gestational age of the patients

The experimental results were computed in order to establish predictive mathematical equations for the normal variation of biochemical investigated markers, during physiological pregnancy (without associated pathology). The results obtained show, interestingly, that all the three biochemical investigated markers exponentially increase with the gestational age in normal pregnancy. The predictive equations that mathematically describe the normal variations of the investigated bio-indoles (melatonin and serotonin) and the predictive equation that describes the normal variation of the oxidative status in physiological pregnancy are presented in Figures 7, 8, 9.

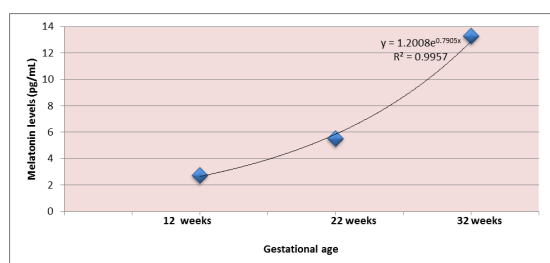


Figure 7.

Predictive equation that describes the normal variation of the serum concentration of melatonin in physiological pregnancy

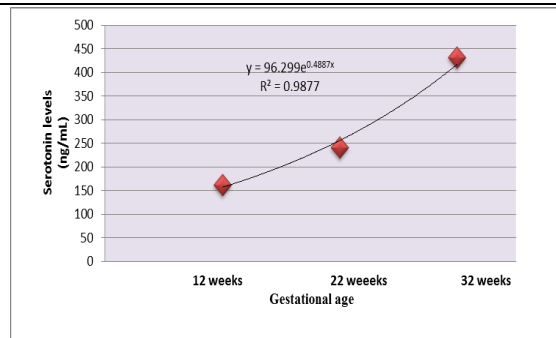


Figure 8.

Predictive equation that describes the normal variation of the serum concentration of serotonin in physiological pregnancy

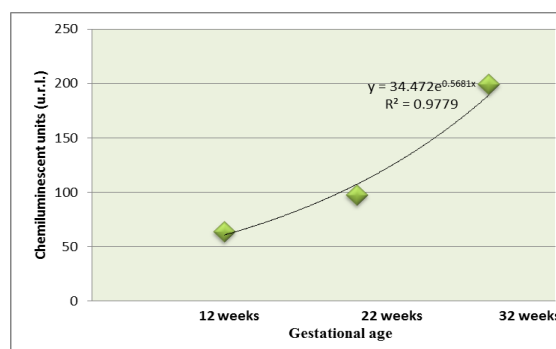


Figure 9.

Predictive equation that describes the normal variation of the serum concentration of oxidative status in physiological pregnancy

Physiological pregnancy is characterized by complex changes in the maternal body: hormonal changes, neuronal, cardiovascular, immunological, adaptive changes, which are based on specific biochemical mechanisms. These molecular mechanisms ensure a normal development and status for both mother and foetus, throughout the gestation period.

The latest researches in this field proved the involvement of melatonin in the sleep-wake rhythm imbalance and the thymic tonus in pregnant women and its protective antioxidant effect on the placenta and thus on the foetus. Thus, presently it is investigated the possibility of using the pineal hormone as biochemical marker for the early diagnosis of gestational diseases as preeclampsia, gestational diabetes, thromboembolism.

Recently it was discovered that the level of circulating serotonin in pregnant women majorly influences the normal development of the fetal brain, the serotonergic neurons of the foetus being identified since 5th week of pregnancy. Thus, a low serum concentration of serotonin into the serum of pregnant women was been positively correlated, statistically significant, to the incidence of autism development in child. Based on these assumptions

published in scientific literature, serotonin may be considered a potential fundamental biochemical marker for the diagnosis of gestational diseases.

Clinical researches showed a positive correlation of the maternal oxidative status with the incidence of some gestational diseases, especially gestational diabetes and preeclampsia. Therefore, the oxidative status of the mother may be a predictive biochemical marker for the occurrence and development of some diseases associated with pregnancy.

Conclusions

The results of the conducted study show, interestingly, that all the investigated biochemical markers: serum concentration of melatonin and of serotonin and the serum level of the oxidative status, exponentially increase with the development of the gestational age, in normal pregnancy.

The experimental results were mathematically processed and we established for the first time in literature, predictive mathematical equations for the normal variation of the investigated biochemical markers, during physiological pregnancy.

In conclusion, the researches described in this paper bring scientific evidences, substantiated by mathematical formulas, to place the investigated parameters in clinical routine, as biochemical predictive markers for the evolution of physiological pregnancy and for the early diagnosis of gestational pathologies.

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