THE CORRELATION BETWEEN CHANGING ESTROGEN AND PROGESTERONE LEVELS AND BLOOD GLUTAMATE LEVELS DURING NORMAL PREGNANCY

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КОРРЕЛЯЦІЯ ЗМІН РІВНЯ ЕСТРОГЕНУ, ПРОГЕСТЕРОНУ ТА ГЛУТАМАТУ В КРОВІ ПРИ НОРМАЛЬНІЙ ВАГІТНОСТІ

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Наше дослідження спрямоване на визначення впливу рівнів прогестерону та естрогену під час вагітності на рівень глутамату крові. Ми відібрали 116 жінок і розділили їх на три групи в залежності від терміну гестації: група 1 – 2 жінки в першому триместрі вагітності, група 2 – жінки у другому триместрі вагітності, група 3 – жінки в третьому триместрі вагітності. Ми одноразово взяли зразки крові у жінок і визначили рівні естрогену, прогестерону, глутамат-піруват трансамінази (ГПТ), глутамат-оксалоацетат трансамінази (ГОТ) та глутамату. Аналізи показали, що вміст глутамату в другому (p <0.001) та третьому (p <0.001) триместрах значно нижче. Дослідження також показало наявність зворотньої кореляції рівня естрогену та прогестерону, та рівня глутамату крові протягом всієї вагітності (p <0.001). З іншого боку, рівні ГПТ І ГОТ не змінювалися під час вагітності, незважаючи на незначне зниження ГПТ в третьому триместрі. Зворотня кореляція була також отримана між підвищеними рівнями естрогену і прогестерону на момент кінцевих стадій вагітностей та рівнем глутамату крові жінок. Подальше зростання рівнів естрогену і прогестерону ледь впливав на рівень глутамату крові після того, як глутамат-редукуючий ефект досягав свого піку. Дані вищевикладеного дослідження демонструють вплив рівнів естрогену та прогестерону на рівень глутамату крові, механізм якого не обумовлений ГОТ/ГПТ конвертуючими механізмами

Ключові слова: естроген; прогестерон; глутамат крові; вагітність; нейропротекція.

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Our investigation aimed to examine the impact of changing estrogen and progesterone levels during normal pregnancy on blood glutamate levels. We recruited 116 pregnant women and partitioned them into three groups based on their gestational ages: group 1 – first-trimester pregnancy, group 2 – second-trimester pregnancy, and group 3 – third-trimester pregnancy. We drew single samples of venous blood from the participating women and analyzed for differing levels of estrogen, progesterone, glutamate-pyruvate transaminase (GPT), glutamate-oxaloacetate transaminase (GOT), and glutamate. Results showed that the second ($p<0.001$) and third trimesters ($p<0.001$) had significantly lower blood glutamate levels. Analyses also revealed the existence of an inverse correlation between blood glutamate levels and estrogen and progesterone levels in the entirety of the pregnancies ($p<0.001$). On the other hand, GOT and GPT levels were unchanged during pregnancy periods, although a slight reduction in GPT was observed during the third trimester. Inverse correlations were also obtained between increased estrogen and progesterone levels during the advanced stages of the pregnancies and blood glutamate levels of the women. Further rises in estrogen and progesterone levels barely impacted blood glutamate levels once the blood glutamate reducing effect reached the maximal point. Findings from this study show that changing estrogen and progesterone levels have an impact on blood glutamate levels, a mechanism that is most probably not facilitated by a GOT/GPT conversion mechanism.

Keywords: estrogen; progesterone; blood glutamate; pregnancy; neuroprotection.

Introduction

Estrogen (E) and Progesterone (P) are female sex hormones whose actions are not limited to tissue-related reproductive functions. E and P reportedly play a role in the development of a variety of body tissues, such as tissues of the central and peripheral nervous systems. These two hormones are allegedly also neuroprotective against various neurodegenerative disorders [12], although the precise mechanism of how either ensures this neuroprotection is still elusive. Hypothetical theories have it that E and P may induce neuroprotection through excitatory neurotransmitter systems, such as glutamate receptors [6] and glutamate transporters [10].

Severe neurological crises are often accompanied by unusually high brain extracellular (ECF) and cerebrospinal fluid (CSF) L-glutamate (glutamate) levels that are neurotoxic and linked to poor neurological outcomes in the event of neurodegenerative disorders in the example of a traumatic brain injury (TBI) and stroke [4]. High levels of glutamate can further increase and become chronic under certain disease circumstances, such as glaucoma, amyotrophic lateral sclerosis (ALS), and HIV-dementia [5, 13]. Excess brain interstitium glutamate levels accelerate the stimulation of glutamate receptors, which causes cell swelling, apoptosis, and neuronal death, all elements of a compromised neurological outcome.

While strong correlations between blood and brain glutamate levels have been established by several investigators [7, 19], other researchers have been quick to point out that reduced blood glutamate levels provided neuroprotection [3, 18, 26-28]. We showed in our previous investigation that blood glutamate levels in male rats decreased when injected with E; we injected these rats with Premarin (a mixture of different estrogens), which resulted in a significant, long-term decrease in blood glutamate levels, correlating with a better neurological outcome [29]. There are suggestions that the glutamate-related mechanism serves as the route for the neuroprotective properties of E and P [2, 9]. E and P could reportedly also decrease the human blood glutamate levels, while there is evidence that levels of glutamate are significantly lower in healthy females of the human population [30]. Also, there is proof that blood glutamate levels correlate inversely with plasma E and P levels in ovulating women during menstruation [25].

Our investigation aimed to investigate the impact of changing E and P levels on blood glutamate levels during normal pregnancy. Fluctuating plasma E and P levels are predictable during normal pregnancy, with the overall levels of both hormones E and P highly elevated in pregnant women relative to their non-pregnant counterparts [21].

We expected that increasing blood glutamate concentrations would reach maximum levels during the first trimester of pregnancy, which coincided with minimal E and P plasma levels. We also anticipated that blood glutamate levels would slowly decrease with increasing plasma E and P levels. We deemed it essential to investigate the possibility of high levels of E and P typically seen during pregnancy impacting blood glutamate levels through a comparable mechanism to that engineering the fluctuation of glutamate during ovulation. Contrary to fluctuating E and P levels noted during the menstrual cycle, E and P levels are known to increase and are usually more pronounced during pregnancy. Our curiosity to this effect was to tell if higher E and P levels during pregnancy affect blood glutamate levels as is the case with lower E and P levels during ovulation. We measured levels of glutamate-pyruvate transaminase (GPT), glutamate-oxaloacetate transaminase (GOT), and glucose to determine their involvement in the possible glutamate-reducing abilities of E and P [24, 28].

Materials and Methods

Soroka University Medical Center, Beer Sheva, Israel was the site of our investigation, which was ratified by the Institutional Review Board. We received written and signed informed consent from each participant before the research, with 116 healthy pregnant women taking part in the study. The benchmark for exclusion included under 18 years candidates, known fetal anomalies, maternal co-morbidities, such as pregnancy-induced hypertension (or pre-eclampsia), renal or hepatic failure, chronic steroid treatment, estrogen or progesterone treatment, anemia (Hb < 12 g/dl), diabetes mellitus, or women with certain metabolic disorders.

Following their stages of gestation, we partitioned the women into 3 groups. First-trimester women (from 6 to 14 weeks) enlisted from candidates seeking elective termination of their pregnancies occupied group 1. Group 2 consisted of second-trimester women (14 +1/7 weeks until 28 weeks) enrolled from candidates seeking elective clinical examinations, such as an ultrasound test. Group 3 was made up of third-trimester women (28 + 1/7 until 42 weeks) drafted from candidates who had pathological fetal presentations, macrosomia, or previous cesarean sections and were seeking elective clinical examinations or elective cesarean sections.

Single samples of venous blood (10 ml, via the antecubital vein) were drawn from the women and subjected to analyses for estrogen, progesterone, glutamate, GPT, and GOT levels. A blood glucose monitoring device, "glucocheck sensor" (Roche, Germany) helped evaluate blood glucose levels immediately after drawing blood from patients. Portions of the collected blood were centrifuged, and the serum analyzed to assess plasma GOT and GPT levels using the fluorescent method and Olympus AU 2700 Assay (Minnesota, USA).

To determine plasma E and P levels, blood samples were again subjected to centrifugation, with the resulting serum analyzed immediately or stored in a freezer at -80º C till it was evaluated with an ADVIA Centaur Estradiol/Progesterone Assay (Bayer, NY, USA), whose principle is based on competitive immunoassay; this allows measurement, by a direct chemiluminescent technology, of the highly specific antibodies produced.

For the evaluation of blood glutamate levels, we added an identical volume of ice-cold 1M perchloric acid (PCA) to whole blood (200 μl aliquot) and then centrifuged at 10000×g for 10 min at 4^0C, deproteinizing it. The resulting pellet was thrown away, leaving the supernatant, whose pH was subsequently adjusted to 7.2 with the use of 2M K_2CO_3; some amounts of the supernatant was stored at -80^0C in case later analyses were required. We then applied Graham and Aprison's fluorometric method [8] to quantify the levels of glutamate as follows: we added a PCA supernatant aliquot (20 μl) to a mix of 0.3 M glycine, 0.25 M hydrazine hydrate buffer (480 μl) and 1N H_2SO_4 and 15 U of glutamate dehydrogenase in 0.2 mM NAD that adjusted the pH to 8.6. The mixture was then incubated, and after a 30-45 min incubation at room temperature, we measured the fluorescence at 460 nm, with 350 nm the excitation mark. The concentrations of glutamate used for the glutamate standard curve were between 0 and 6 μM. Every measure carried out was duplicated.

For statistical analyses, we used the SPSS 17 package (SPSS Inc., Chicago, USA). We used the two-tailed t-test for equality of means and Levene's Test for Equality of Variances to compare glutamate, glucose, GOT, and GPT levels between various groups. $p < 0.05$ was considered significant and $p < 0.001$ highly substantial. The presentation of data is means ± SEM.

Results
Our research included 116 pregnant women in all: 43 of the pregnancies were first-trimester, 43 were third-trimester, and only 30 were second-trimester because we found it hard enrolling healthy pregnant women during this trimester. Figure 1 contains changes in the level of blood glutamate during al three trimesters of pregnancy. Blood glutamate levels in second-trimester pregnancies decreased significantly (98.3 ± 6 μM/L) in comparison to those in first-trimester pregnancies (167 ± 13 μM/L) (P<0.0001). Blood glutamate levels in third-trimester pregnancies were at comparable levels to those in second-trimester pregnancies (88.9 ± 8 μM/L) but were substantially lower than those in first-trimester pregnancies (P<0.0001).

Figure 2 is a representation of Blood E and P levels. Plasma E levels increased significantly in second-trimester pregnant women (8158 ± 1030 pg/mL) in comparison to the levels in first-trimester pregnant women (1780 ± 190 pg/mL) (P<0.0001). Plasma E levels rose further in third-trimester pregnant women, reaching 20735 ± 1620 pg/mL (P<0.0001). Similar changing patterns were observed with regards to P levels, with significant increases noted in second-trimester pregnant women (65 ± 5 ng/mL) when compared to levels in first-trimester pregnant women (23 ± 2 ng/mL) (P<0.0001). As
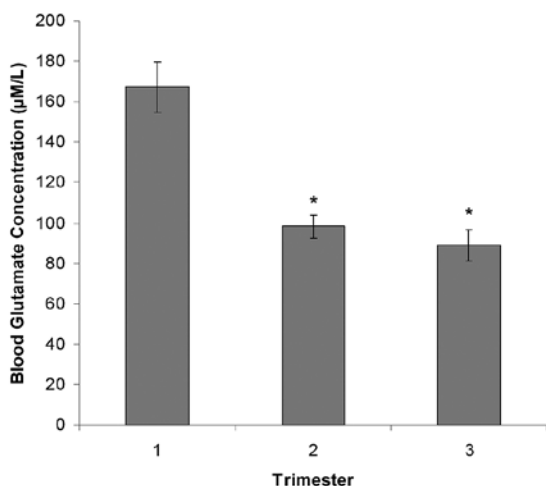
Figure 1. Absolute blood glutamate concentrations observed during each pregnancy trimester. A comparison between the first trimester (167 ± 13 µM/L), second trimester (98.3 ± 6 µM/L; P<0.0001; *) and third trimester (88.9 ± 8 µM/L; P<0.0001; *) revealed that blood glutamate levels were significantly lower in the latter two trimesters, which were comparable to each other. Data representation are shown as mean ± SEM.
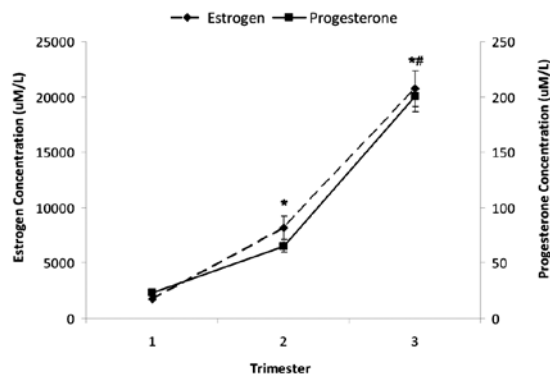

Figure 2: Plasma estrogen and progesterone concentrations observed during each trimester of pregnancy. Compared with the first trimester, concentrations of plasma estrogen (1780 ± 190 pg/mL) and progesterone (65 ± 5 ng/mL) were significantly higher during the second trimester (8158 ± 1030 pg/mL and 65 ± 5 ng/mL respectively; P<0.0001; *) and third trimester (20735 ± 1620 pg/mL and 23 ± 2 ng/mL respectively; P<0.0001; *). There was a further increase in estrogen and progesterone observed in the third trimester when compared to the second trimester (P<0.0001; #). The data are presented as mean ± SEM.

with plasma E levels, quantities of Plasma P increased further in third-trimester pregnant women, reaching 200 ± 14 ng/mL (P<0.0001).

	Trimester		
	1	**2**	**3**
Glucose (mg/dL)	86 ± 2	89 ± 4	80 ± 2
GOT (IU/L)	21 ± 1	20 ± 1	20 ± 1
GPT (IU/L)	19 ± 2	14 ± 1	12 ± 1*

Table 1 displays values for blood glucose, plasma GOT, and plasma GPT during all three trimesters of pregnancy. The levels of blood glucose or GOT stayed constant for the entirety of pregnancies and were comparative among all groups. GPT levels did stay constant during trimesters one and two but diminished significantly by the third pregnancy trimester in comparison to first-trimester levels (P<0.01).

Discussion

Our main finding showed that an inverse correlation exists between increasing E and P levels during advanced stage pregnancies and the blood glutamate levels of the pregnant women. Increasing E and P levels in second-trimester pregnancies were involved with significant decreasing blood glutamate levels, even though the continuing rise in E and P levels in third-trimester pregnant women did not trigger further substantial reductions in blood glutamate levels.

Another primary outcome of our research was the fact that reduced blood glutamate levels were not a sign of rising blood GOT or GPT levels. This finding suggests that if increasing E and P levels can lower blood glutamate amounts, the phenomenon does not include oxaloacetate- and pyruvate-driven GOT and GPT, respectively, converting glutamate to 2-ketoglutarate (inactive form).

E and P reportedly act as great neuroprotectors in several neurodegenerative disorders [14]. An E treatment [9] and pre-treatment [1, 9] of a middle cerebral artery occlusion (MCAO)-induced neurological damage has been shown to lessen the injury. Furthermore, the occurrence of Alzheimer's disease in postmenopausal women reportedly is diminished by E-replacement therapy [11]. According to one finding, E can also delay the onset of clonic seizures caused by kainic acid and cut deaths related to seizures in rats [22]. Still, another finding reports P as neuroprotective during an ischemic stroke [1] and TBI in rats [23].

Despite the continuous assertion that E and P can and serve as neuroprotectors, no elucidation of the exact mechanism of how this assertion works had been forthcoming. Well demonstrated, though, is the fact that glutamate is involved in synthesizing and producing hormones like gonadotropin-releasing hormone (GnRH) [15] and E through hypothalamic neuron-bound precursors of cholesterol [2] upon exposure of in-vitro hypothalamus to glutamate. Increased levels of E subsequently triggers a feedback-engineered down-regulation of concomitant raised plasma glutamate levels in the brain. E and P may, therefore, have an essential role in auto-regulating glutamate levels in the brain and blood.

Very little is known to date with regards to the effects of sex differences on the levels of glutamate. Reports have it that levels of blood glutamate are in women lower than those in men among healthy individuals [30], supported by Stover and Kempski's investigation, which showed that male patients had higher glutamate levels than their female counterparts, while also establishing baseline glutamate levels in female patients after they were all given isoflurane during neurosurgical procedure [17]. Despite not performing this procedure in the present study, our finding is consistent with claims that decreasing levels of blood glutamate are capable of stimulating neuroprotection in women. We also demonstrated recently that naïve and TBI male rats that received E via injection had lesser blood glutamate levels and improved neurological outcomes [29].

Results from this study indicate that high maternal E and P levels in advanced pregnancies correlated with lower blood glutamate levels. We anticipated a continuous reduction in the levels of blood glutamate as pregnancies advanced from second to third trimesters, given that E and P levels continuously increased. However, the reduction in blood glutamate levels was less distinct in third-trimester pregnant women, although E and P levels at this stage were significantly higher than those in second-trimester pregnancies. We, therefore, postulate that the attainment of the highest blood glutamate reducing effect means that further increases in E and P levels exert minimal impacts

on the levels of glutamate in the blood. Increased P levels have, in the past, been shown to cause a decrease in the levels of glutamate in the blood in a dose-response mode likened to a bell-shape, with this impact also noted to die down at both higher and lower concentrations [16]. This explanation could be given to account for why glutamate reduction in the blood during third-trimester pregnancies was not as significant as it was during the second trimester. Hence, it would be of some assistance taking into consideration our finding when deciding on the suitable theoretical E and P doses for treatment as discoveries of new therapeutic modalities are put forward for the improvement of neurological outcomes in the event of an acute neurological attack.

The maintenance of GOT and GPT at constant levels for the entire pregnancy period suggested the existence of a different E and P-mediated blood glutamate reducing mechanism from the one that ensures that glutamate is converted to its inactive form. This result affirms interpretations made by previous investigators that women have lower blood glutamate, GOT, and GPT levels than men [30], and the levels of GOT and GPT stay unchanged for the entirety of the menstrual cycle [25] and at different pregnancy stages [20].

Given that GOT and GPT are scavengers of blood glutamate, it would be anticipated that elevated levels in women are required to ensure lower blood glutamate levels. The thought of women having both diminished blood glutamate levels and reduced GOT and GPT levels would, thus, sound contradictory. Our observation may, hence, only be attributed to E or P impacting female glutamate levels via an independent GOT/GPT-scavenging mechanism. In this scenario, a lesser amount of GOT or GPT would be sufficient to trigger the conversion of the diminished glutamate levels to inactive forms. Another justification for our observation may also lie in the fact that E and P are capable of playing roles in the rise of plasma oxaloacetate and pyruvate levels that operate as GOT and GPT co-enzymes, respectively. If so, then the equation for the Michaelis-Menten enzyme rate would predict lower GOT and GPT levels necessary for the conversion of equal amounts of glutamate to 2-ketoglutarate, which would ensure sustainability of lower blood glutamate levels [26, 28].

The one major drawback of our research would be our measuring of glutamate levels only in the blood and not in the brain, which would have been a benediction of our previous investigation in rats, where we revealed that there is a strong correlation between low levels of glutamate in the blood and low levels of glutamate in the ECF of the brain [7, 19]. However, there is also a correlation between low levels of blood glutamate and an improved neurological outcome post-TBI as reported in the past [26-28]. In this regard, we elected to go for a less invasive approach to measure glutamate levels, which is in the blood, instead of the overly intrusive brain ECF-related evaluation. Our theory, therefore, based on our findings, stipulates that the blood glutamate-reducing hormones, E and P, could also cause brain glutamate reductions. Nevertheless, additional studies are required to back this concept.

The second drawback to our research would be the need for establishing a direct association between E, P, and glutamate levels and its impact on neurological outcomes post-acute brain attacks. To that effect, more in-depth studies are needed. Furthermore, we are still to decipher the precise mechanisms for the mediation of blood glutamate reduction by E and P.

Nuances notwithstanding, we were able to provide useful insights into the impactful role E and P occupy during blood glutamate reduction, which is a significant step

to understanding the blood glutamate-regulating physiological mechanisms and could aid in the establishment of new therapeutic modalities that seek to better neurological outcomes post-acute neurological attacks.

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