UDC 612.63.01+618.2/.3+664.644.5+577.151.02
DOI: 10.56871/RBR.2024.19.88.003

STUDY OF THE ACTIVITY OF DIGESTIVE ENZYMES OF BIOLOGICAL FLUIDS IN WOMEN DURING PREGNANCY

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For citation: Kolodkina EV, Lytaev SA. Study of the activity of digestive enzymes of biological fluids in women during pregnancy. Russian Biomedical Research. 2024;9(4):16–22. DOI: https://doi.org/10.56871/RBR.2024.19.88.003

Received: 19.09.2024 **Revised: 05.11.2024** **Accepted: 17.12.2024**

Abstract. ***Introduction.*** The hydrolytic activity of biofluids during pregnancy has been studied. It was found that their content in blood serum, saliva, urine and coprofiltrate dynamically change from the end of pregnancy to the early postpartum period. It has been shown that digestive enzymes play an important role in the formation of the Mother–fetus interaction. ***The purpose of the work*** — to study the activity of digestive enzymes in peripheral blood serum, urine, saliva, coprofiltrate, amniotic fluid, umbilical cord blood in women during pregnancy after childbirth, to compare with that content in non-pregnant women. ***Materials and methods.*** The material for the study was taken from non-pregnant (n=45) and pregnant (n=82) women — new mothers aged from 18 to 35 years (2–3 days after childbirth). The dynamics of changes in the activity of hydrolases in biological fluids was studied. ***Results.*** A change in the activity levels of pepsinogen, amylase, alkaline phosphatase and lipase in biological fluids in women during pregnancy and in the postpartum period compared with those in non-pregnant women was revealed. ***Conclusions.*** In pregnant women, by the end of pregnancy, there is an increase in the activity of all hydrolases in blood serum, an increase in the activity of pepsinogen and lipase in urine, a significant increase in amylase in saliva and a decrease in the proteolytic and alkaline phosphatase activities of the coprofiltrate. The homogenate of placenta, umbilical cord blood and amniotic fluid is characterized by the highest activity of alkaline phosphatase.

Keywords: pregnancy, digestive enzymes, enzyme homeostasis, biological fluids, amniotrophic nutrition

ИЗУЧЕНИЕ АКТИВНОСТИ ПИЩЕВАРИТЕЛЬНЫХ ФЕРМЕНТОВ БИОЛОГИЧЕСКИХ ЖИДКОСТЕЙ У ЖЕНЩИН ВО ВРЕМЯ БЕРЕМЕННОСТИ

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Для цитирования: Колодкина Е.В., Лытаев С.А. Изучение активности пищеварительных ферментов биологических жидкостей у женщин во время беременности**.** Российские биомедицинские исследования. 2024;9(4):16–22. DOI: https://doi.org/10.56871/RBR.2024.19.88.003

Поступила: 19.09.2024 **Одобрена: 05.11.2024** **Принята к печати: 17.12.2024**

Резюме. ***Введение.*** Изучена гидролитическая активность биологических жидкостей при беременности. Установлено, что их содержание в сыворотке крови, слюне, моче и копрофильтрате динамически меняется от конца беременности до раннего послеродового периода. Показано, что пищеварительные ферменты

играют важную роль в формировании взаимодействия «мать–плод». ***Цель исследования*** — изучить активность пищеварительных ферментов в сыворотке периферической крови, моче, слюне, копрофильтрате, амниотической жидкости, крови пуповины у женщин при беременности и после родов, сравнить с таковым содержанием у небеременных женщин. ***Материалы и методы.*** Материал для исследования брался у небеременных (n=45) и беременных (n=82) женщин — родильниц в возрасте от 18 до 35 лет (2–3-й день после родов). Изучалась динамика изменения активности гидролаз в биологических жидкостях. ***Результаты.*** Выявлено изменение уровней активности пепсиногена, амилазы, щелочной фосфатазы и липазы в биологических жидкостях у женщин при беременности и в послеродовый период по сравнению с показателями у небеременных женщин. ***Выводы.*** У беременных женщин к концу беременности наблюдается повышение активности всех гидролаз в сыворотке крови, увеличение активности пепсиногена и липазы в моче, существенное повышение амилазы в слюне и снижение протеолитической и щелочно-фосфатазной активностей копрофильтрата. Гомогенат плаценты, пуповинная кровь и амниотическая жидкость отличаются наиболее высокой активностью щелочной фосфатазы.

Ключевые слова: беременность, пищеварительные ферменты, ферментный гомеостаз, биологические жидкости, амниотрофное питание

INTRODUCTION

Different mechanisms are involved in the homeostasis of pepsinogen, amylase, alkaline phosphatase, and lipase, among which are the secretion and excretion of digestive gland hydrolases [5–8, 13, 16].

At present, it has been confirmed that digestive gland hydrolases are widely distributed in the biological media of the organism, which, being increted into blood and lymph, are distributed according to the selective permeability of histohematic barriers, including the uteroplacental barrier, which supplies hydrolases to the amniotic fluid [1, 11, 14, 17–19, 21].

Digestive hydrolases are of particular importance in the system “maternal organism — placenta, amniotic fluid — fetus” [2–5, 8, 9].

Pregnancy establishes a relationship between the maternal body and the growing fetus. The fetus absorbs nutrients with amniotic waters that are hydrolyzed to monomers in the gastrointestinal tract (GIT) of the developing organism by enzymes that are recreted into the aquafetal environment (autolytic digestion) [10, 13, 15, 16, 20, 21].

The “fate” of secreted digestive enzymes in pregnant women, changes in the levels of enzyme activity in biological fluids throughout pregnancy and in the postpartum period have not been fully studied [12, 19, 20].

AIM

To study the activity of digestive enzymes in peripheral blood serum, urine, saliva, coprofiltrate, amniotic fluid, umbilical cord blood in pregnant and postpartum women and compare it with that in non-pregnant women.

MATERIALS AND METHODS

The study was conducted on the basis of the St. Petersburg State Budgetary Health Care Institution “Maternity Hospital No. 6 named after Prof. V.F. Snegirev”.

The material for the study was taken from non-pregnant (n=45) and pregnant (n=82) women. Maternity patients aged from 18 to 35 years.

The content and activity of enzymes (pepsinogen, amylase, lipase and alkaline phosphatase) were studied in peripheral blood serum, urine, oral fluid, coprofiltrate, amniotic fluid, umbilical cord blood in pregnant women at 39–40 weeks of pregnancy, in women on the 2–3rd day after delivery, and once in the control group.

All patients were informed about the purpose and methods and gave written voluntary informed consent to participate in the study (protocol No. 0608-23 of 07.08.2023 of the ethical committee meeting).

Total proteolytic activity was determined at low pH values of 1.5–2.0 by spectrophotometric (tyrosine) Kunitz-Northrop method. Amylolytic activity was determined by amyloclastic method according to Caraway. Lipolytic activity was determined by unified method using olive oil as a substrate. Alkaline phosphatase activity was determined by standard constant time method using a set of reagents from Lahema diagnosticum (Czech Republic).

Differences between the groups by the level of the studied signs were evaluated using the Mann-Whitney U-criterion. Differences were considered significant at the probability of error $p < 0.05$.

Statistical processing of the obtained data was carried out in Microsoft Excel 2003 spreadsheet environment, as well as Primer of biostatistics 4.03 and SPSS 11.0 programs.

RESULTS

As a result of these studies, it was revealed that the blood serum of pregnant women at 39–40 weeks of pregnancy contains 1.5–2 times more hydrolases than that of non-pregnant women (Table 1).

On the 2–3rd day after delivery in women the content of enzymes in the blood serum decreases, but remains higher than in the examined women of the control group. Apparently, this is due to the increased secretion of hydrolases in women during pregnancy [5, 6].

The study of digestive enzyme activity in the urine of the examined women revealed the following changes (Table 2).

At 39–40 weeks of pregnancy, the level of proteolytic and lipolytic activity of urine in women is significantly higher than in non-pregnant women ($p < 0.05$). On the 2–3rd day after delivery in women giving birth, there is a significant decrease in the enzymatic activity of this biological fluid for all the studied indicators, regardless of the increase of these enzymes in the antenatal period.

When studying the activity of digestive enzymes in saliva, it was revealed that the saliva of pregnant women has greater proteolytic activity than that of patients in the control group (Table 3).

The indices of pepsinogen in saliva decreased in postpartum women in 1.8 times ($p < 0.05$) compared to those of

Table 1

Indicators of the activity of digestive enzymes in the blood of control group individuals, women at 39–40 weeks of pregnancy and postpartum women

Таблица 1

Показатели активности пищеварительных ферментов в крови у лиц контрольной группы, женщин на 39–40-й неделе беременности и родильниц после родов

Контингент исследуемых / Contingent of the studied	Пепсиноген, тир. ед/мл / Pepsinogen, tyr. units/ml	Амилаза, ед/мл / Amylase, units/ml	Щелочная фосфатаза, ед/мл / Alkaline phosphatase, units/ml	Липаза, ед/мл / Lipase, units/ml
1. Контрольная группа / Control group (n=45)	58,2±1,1	13,5±0,8	722,1±50,6	18,1±0,7
2. Беременные на 39–40-й неделе беременности / Pregnant women at 39–40 weeks of pregnancy (n=82)	60,2±4,1	23,0±1,7**	1182,3±106,4*	34,6±1,7**
3. Родильницы на 2–3-й день после родов / Maternity hospitals 2–3 days after delivery (n=82)	44,9±1,1*	19,6±0,8*	868,4±92,3	26,4±1,2**

Note: the reliability of differences with the indicators of the control group: * — p <0,001; ** — p <0,05.
Примечание: достоверность различий с показателями контрольной группы: * — p <0,001; ** — p <0,05.

Table 2

Indicators of the activity of digestive enzymes in the urine of individuals in the control group, women at 39–40 weeks of pregnancy and postpartum women

Таблица 2

Показатели активности пищеварительных ферментов в моче у лиц контрольной группы, женщин на 39–40-й неделе беременности и родильниц после родов

Контингент исследуемых / Contingent of the studied	Пепсиноген, тир. ед/мл / Pepsinogen, tyr. units/ml	Амилаза, ед/мл / Amylase, units/ml	Щелочная фосфатаза, ед/мл / Alkaline phosphatase, units/ml	Липаза, ед/мл / Lipase, units/ml
1. Контрольная группа / Control group (n=45)	4520,3±320,4	64,1±1,2	428,6±29,0	20,6±1,2
2. Беременные на 39–40-й неделе беременности / Pregnant women at 39–40 weeks of pregnancy (n=82)	9650,1±504,1**	67,2±1,4	410,9±36,2	41,2±1,6**
3. Родильницы на 2–3-й день после родов / Maternity hospitals 2–3 days after delivery (n=82)	3698,5±278,8*	41,2±0,9*	240,4±17,8**	27,5±1,5**

Note: the reliability of differences with the indicators of the control group: * — p <0,001; ** — p <0,05.
Примечание: достоверность различий с показателями контрольной группы: * — p <0,001; ** — p <0,05.

Table 3

Indicators of the activity of digestive enzymes in saliva in the control group, women at 39–40 weeks of pregnancy and postpartum women

Таблица 3

Показатели активности пищеварительных ферментов в слюне у лиц контрольной группы, женщин на 39–40-й неделе беременности и родильниц после родов

Контингент исследуемых / Contingent of the studied	Пепсиноген, тир. ед/мл / Pepsinogen, tyr. units/ml	Амилаза, ед/мл / Amylase, units/ml	Щелочная фосфатаза, ед/мл / Alkaline phosphatase, units/ml	Липаза, ед/мл / Lipase, units/ml
1. Контрольная группа / Control group (n=45)	1520,9±247,6	2385,3±264,7	215,6±22,3	64,8±7,0
2. Беременные на 39–40-й неделе беременности / Pregnant women at 39–40 weeks of pregnancy (n=82)	2612,9±218,1*	4781,6±423,8*	361,8±30,2	121,1±11,6*
3. Родильницы на 2–3-й день после родов / Maternity hospitals 2–3 days after delivery (n=82)	1463,3±221,6	3109,0±294,2**	475,3±31,6**	70,9±5,4

Note: the reliability of differences with the indicators of the control group: * — p <0,001; ** — p <0,05.
Примечание: достоверность различий с показателями контрольной группы: * — p <0,001; ** — p <0,05.

Table 4

Indicators of the activity of digestive enzymes in coprofiltrate in the control group, women at 39–40 weeks of pregnancy and postpartum women

Таблица 4

Показатели активности пищеварительных ферментов в копрофильтрате у лиц контрольной группы, женщин на 39–40-й неделе беременности и родильниц после родов

Контингент исследуемых / Contingent of the studied	Пепсиноген, тир. ед/мл / Pepsinogen, tyr. units/ml	Амилаза, ед/мл / Amylase, units/ml	Щелочная фосфатаза, ед/мл / Alkaline phosphatase, units/ml	Липаза, ед/мл / Lipase, units/ml
1. Контрольная группа / Control group (n=45)	442,2±20,5	19,5±0,8	6220,4±248,0	320,8±12,6
2. Беременные на 39–40-й недели беременности / Pregnant women at 39–40 weeks of pregnancy (n=82)	153,8±10,9*	44,4±3,9*	2236,6±158,6*	344,4±17,2
3. Родильницы на 2–3-й день после родов / Maternity hospitals 2–3 days after delivery (n=82)	315,3±16,8**	18,3±0,9	3229,2±122,1**	324,3±13,6

Note: the reliability of differences with the indicators of the control group: * — p <0,001; ** — p <0,05.
Примечание: достоверность различий с показателями контрольной группы: * — p <0,001; ** — p <0,05.

pregnant women at 39–40 weeks of pregnancy, which corresponds to the protein-producing function in the "mother–fetus" system (Table 3).

Amylolytic activity of saliva had a significant increase by 39-40 weeks of pregnancy (2-fold, p <0.001) in women compared to controls. On the 2–3rd day after delivery, amylase activity decreased, not reaching the values in non-pregnant women.

Similar dynamics was observed with respect to salivary lipase activity, with an increase in the enzyme level at the end of pregnancy and a decrease in the postpartum period.

There was a significant increase in alkaline phosphatase activity of saliva at 39–40 weeks of pregnancy by 1.9 times (p <0.001) in women compared with control parameters, with a further increase in the level of the enzyme in women on the 2–3rd day after delivery.

Indices of activity of digestive enzymes in coprofiltrate in the studied groups of women are presented in Table 4. There was a significant decrease in proteolytic and alkaline phosphatase activity of coprofiltrate in pregnant women on the 39–40th week of pregnancy and in laboring women on the 2–3rd day after delivery compared to the control group.

Amylolytic activity of coprofiltrate in pregnant women reaches values twice higher than in the control group by the end of pregnancy. After delivery, it becomes lower than the initial level and indicators of non-pregnant women.

Table 5

Indicators of the activity of hydrolases of amniotic fluid, umbilical cord blood, and placenta

Таблица 5

Показатели активности гидролаз амниотической жидкости, плаценты и пуповинной крови

Гидролитическая активность / Hydrolytic activity	Амниотическая жидкость / Amniotic fluid	Гомогенат плаценты / Placenta homogenate	Пуповинная кровь / Umbilical cord blood
Амилолитическая, ед/мл / Amylolytic, units/ml	16,3±0,7	9,2±0,5	36,7±4,5**
Щелочно-фосфатазная, ед/мл / Alkaline phosphatase, units/ml	3287,6±174,0	6906,2±208,1*	1282,1±316,2
Общая протеолитическая, тир. ед/мл / Common proteolytic, tyr. units/ml	566,4±39,1	759,0±25,5*	104,1±46,9*
Липолитическая, ед/мл / Lypolytic, units/ml	232,1±17,8	212,3±36,4	167,7±12,3**

Примечание: достоверность различий с показателями амниотической жидкости: * — p <0,001; ** — p <0,05.

Note: the reliability of differences with the indicators of the amniotic fluid: * — p <0,001; ** — p <0,05.

There were no significant differences in the lipolytic activity of coprofiltrate in women at the end of pregnancy and after childbirth compared to that in non-pregnant women.

Pregnancy ensures the circulation of digestive hydrolases in the system “maternal organism — placenta, amniotic fluid — fetus” for their subsequent participation in hematotrophic nutrition and autolytic digestion of the fetus [5, 6, 15].

Amniotic fluid and umbilical cord blood have high hydrolytic activity. The extract from placenta contains hydrolases in a greater amount, taking into account the fivefold dilution of the homogenate (Table 5).

Thus, amylolytic activity in umbilical cord blood is 2 times higher than in amniotic fluid. The total proteolytic activity was significantly higher in the extract from placenta and amniotic fluid than in umbilical cord blood, indicating the concentrating ability of the placenta and amnion. The same pattern was observed with respect to lipase activity. At the same time, the content of alkaline phosphatase was particularly high in the extract from the placenta, and the amniotic fluid and cord blood contained almost as much of the enzyme as the maternal blood plasma before labor.

Thus, hydrolases secreted by digestive glands can participate in the organization of creator communication, formation of functional blocks of different purposes and functional systems of trophic and transport support for interorgan and interorganism interaction in the system “maternal organism — placenta, amniotic fluid — fetus”.

DISCUSSION

In the biological fluids of the system “mother — fetus — newborn — infant” selectively specific digestive enzymes, secreted in the maternal body, are distributed, detected by proteolytic, amylolytic, lipolytic, alkaline-phosphatase activities of blood plasma (serum), saliva, urine, coprofiltrate and amniotic fluid.

Our studies revealed a slight increase in pepsinogen activity in blood serum in pregnant women at 39–40 weeks of pregnancy with a subsequent decrease in the activity of the enzyme in laboring women on the 2–3rd day after delivery.

In pregnant women there was an increase in amylolytic, alkaline-phosphatase and lipolytic activities of blood serum in the III trimester compared to the indicators of the control group. In the postpartum period, enzyme activity decreased, not reaching the values of non-pregnant women.

The homeostasis of hydrolases synthesized by digestive glands is maintained by a system of interacting mechanisms, among which the renal excretion pathway is considered to be the most significant [8].

According to G.F. Korotko, the kidneys excrete a very significant amount of increted enzymes, while other pathways of enzyme excretion and degradation cannot compensate for their renal excretion from the body [8].

Our studies showed that the proteolytic activity of urine steadily increased towards the end of pregnancy, which is associated with increased enzyme excretion in urine and saliva.

Similar dynamics had lipolytic activity of urine, which increased by the end of pregnancy in all women who gave birth and remained higher than in non-pregnant women.

At 39–40 weeks of pregnancy, an increase in amylolytic and a decrease in alkaline phosphatase activity of urine was observed in women compared with the control group [5, 8].

In all pregnant women, the recreation of enzymes (amylase, lipase and pepsinogen) by the salivary glands is directly related to gestational age, with an increase in enzyme activity at the end of pregnancy and a decrease after delivery. Alkaline phosphatase activity increased significantly in the dynamics of pregnancy, with the greatest increase after delivery.

Excretory-recretory origin of hydrolytic activity of coprofiltrate is explained by the fact of detection of amylase, pepsinogen, lipase and alkaline phosphatase in feces. At 39–40 weeks of pregnancy, amylolytic activity increased in all women, while pepsinase and alkaline phosphatase activity decreased almost 3-fold ($p < 0.001$) compared to the values of non-pregnant women. There were no significant differences in lipolytic activity of coprofiltrate in pregnant women and laboring women.

The amnioplacental barrier is involved in the selective accumulation of digestive enzymes in amniotic fluid and in the placenta itself [5, 6, 15, 19].

We found that in placenta homogenate and amniotic fluid the content of enzymes is quite significant. Umbilical cord blood in comparison with placenta homogenate and amniotic fluid contains less enzymes: there is 6–7 times lower pepsinogen activity than in placenta, and 4–5 times lower than in amniotic fluid.

Our data indicate that the homeostasis of hydrolases is maintained due to their recreation and excretion from the body. In pregnancy, there is a dynamic change in the indicators of enzymatic activity of biological fluids, while homeostatic parameters are maintained at the level necessary for its development.

CONCLUSION

1. At the 39-40th week of pregnancy, women showed an increase in proteolytic, amylolytic, alkaline phosphatase and lipolytic activities of blood serum compared to the control group.

2. A significant increase in pepsinogen and lipase activity in urine was observed in women toward the end of pregnancy, followed by their decrease in postpartum women.

3. The greatest changes in saliva of pregnant women were observed with respect to amylase, the activity of which remained high in postpartum women.

4. There was a significant decrease in proteolytic and alkaline phosphatase activities of coprofiltrate in pregnant women at 39-40 weeks of pregnancy and in postpartum women at 2-3 days after delivery compared to the control group.

5. High activity of alkaline phosphatase is noted in placenta homogenate, umbilical cord blood and amniotic fluid. Total proteolytic activity is most pronounced in placenta homogenate and amniotic fluid.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

Funding source. This study was not supported by any external sources of funding.

Consent for publication. Written consent was obtained from the patient for publication of relevant medical information within the manuscript.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Информированное согласие на публикацию. Авторы получили письменное согласие пациентов на публикацию медицинских данных.

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