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Lactate plasma level as a potential biomarker in early diagnosis of ectopic pregnancy: A case-control survey

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Abstract

Introduction: A novel metabolomics survey proposed lactic acid as a diagnostic biomarker to detect ectopic pregnancy (EP). Here we investigate the plasma level of lactate for early diagnosis of EP as a potential biomarker.

Methods: In a case-control study, the reproductive aged women with definite tubal EP (6–10 weeks' gestation), referred to our department during 2021–2022, considered as case group, and women with normal singleton pregnancy in the same gestational age as control group. After informed concept, demographic data (maternal and gestational age and parity) recorded and 5 mL venous blood samples were taken to detect the lactate plasma level. The data analyzed using SPSS software ver22.

Results: Finally, 95 participations (50 in case and 45 in control group) enrolled. The clinical results showed that the most of case group were aged more than 35 years old with had higher parity and body mass index, but, no statistically significant difference showed up. On the other hand, although the lactate level was slightly higher in women with EP, but, the plasma lactate level did not statistically differ between the two study groups. Also, the logistic regression showed no relationship between the demographic variables and the lactate plasma level.

Conclusion: It seems that the plasma level of lactate cannot be a diagnostic biomarker for EP.

KEYWORDS

biomarker, diagnosis, ectopic pregnancy, lactate plasma level

1 | INTRODUCTION

Ectopic pregnancy (EP) is one of the most important obstetric emergencies and one of the most common cause of maternal death in the first trimester. As World Health Organization has declared, 4.9% of maternal deaths are related to EP.[1,2] The prevalence of EP is increasing worldwide. An Iranian meta-analysis reported a prevalence rate of EP before 2006 as 1.9 and after 2006 as 3.7 in 1000 pregnancies.[3]

The diagnosis of EP is based on a combination of β-human chorionic gonadotropin (β-hCG) titer and vaginal ultrasonography.[4]

Unfortunately, available diagnostic tests are with a high rate of false positive and false negative results, so, it seems necessary to identify new screening tests such as a more accurate biomarker that is less dependent on individual expertize, and results in early diagnosis and treatment, and decrease the risk of maternal death. The new metabolomics may result in new diagnostic biomarkers which is a unique biological indicator represents a countable, evaluable, and comparable diagnostic clue.[5]

Nowadays, various biomarkers have been proposed to increase the accuracy of EP diagnosis, such as progesterone plasma levels, estradiol, vascular endothelial growth factor A, Inhibin-A, and Activin-A,[6–12] but, no biomarker has been identified as a definite diagnostic clue for EP.[13] There are some proposed biomarkers for muscle damage, trophoblast dysfunction, angiogenesis, and defective tubular migration, but, these results showed low validity.[6,14–17]

The results of the first and only metabolomics study to investigate EP showed that the two metabolomics (lactic acid and acetic acid) can be acceptable biomarkers with high accuracy for the diagnosis of intrauterine pregnancy, although the highest diagnostic power for EP in the VIP plot (variable importance in projection) has been related to lactic acid.[18]

During embryogenesis, glucose is the main source of nutrition for blastocysts, and about 90% of the glucose consumption is from the nonoxidative pathway results in lactic acid production and creates the acidic environment in early stages of pregnancy which is necessary for embryo implantation.[18,19] On the other hand, in the early stages of pregnancy, lactic acid is effective in trophoblastic invasion and angiogenesis.[20] Also, it is necessary to suppress the maternal immune system in order not to reject the fetus as a signal transmitter for deciduae immune cells results in fetal allogeneic immune tolerance. Despite these critical role, the lactate level during pregnancy is in a range of 5.5–14.5 mg/dL or 0.1–61.61 mmol/L, which is near to normal![20,21,22]

Early diagnosis of EP is a major key to reduce maternal mortality and morbidities and unfortunately, there is no clinical evidence of lactic acid as a diagnostic biomarker, here we investigate the plasma level of lactate in the early diagnosis of EP. To our best knowledge, this is the first survey on this topic and if there is a clear difference with normal pregnancy, it will be suggested as a biomarker for accurate and early diagnosis of EP.

2 | MATERIALS AND METHODS

In a case-control study, the reproductive aged women with definite tubal EP (6–10 weeks' gestation), referred to our department during 2021–2022, considered as case group, and women with normal singleton pregnancy in the same gestational age confirmed by ultrasonography (a visible gestational sac with yolk sac or fetal heartbeat) as control group. The diagnosis of EP was stablished through ultrasound and serial measurement of plasma β-hCG levels as gold standard.

- **Exclusion criteria:** Gestational age > 10 weeks, hemodynamics instability, medical treatment in current pregnancy, active pelvic inflammatory disease, nontubal EP such as (cornea, cervix or ovary), history of autoimmune diseases, rheumatic disease, malignancy, hypertriglyceridemia (TG > 1400 mg/dL), hemolysis, active liver disease, ascorbic acid supplementation, tubal rupture, and BMI above 30.
- **Sampling:** Based on the study of Onur Turkoglu et al.,[12] the sample size was estimated to be 50 people in each group (with a standard error of 0.05, specificity = 0.966, $d = 0.05$, and prevalence = 0.0037[23]).
- **Study protocol:** After informed concept, demographic data (maternal and gestational age and parity) recorded and 5 mL venous blood samples were taken in a state of complete rest and without tourniquet, to detect the lactate plasma level. The blood sample mixed in ethylenediaminetetraacetic acid-coated tubes and plasma was separated after 15 min. During sampling, we prevented of the contamination of samples with saliva or sweat. Plasma lactate level and demographic information of these people including age, BMI, and gestational age, parity were recorded. The plasma level of lactate was measured by enzymatic/colorimetric method by Birfax Fars lactate kit (BXC0622). The normal range of lactate in a venous blood sample is 5.5–14.5 mg/dL (which is equivalent to 0.61–1.61 mmol/L), and the normal value in pregnancy is less than 2 mmol/L.
- **Data analysis**: The data analyzed using SPSS software ver22 through Mann-Whitney and logistic regression tests. The receiver operating characteristic curve was used to investigate the diagnostic value of plasma lactic acid level as a biomarker in the early diagnosis of EP. A significance level of $p < 0.05$ was considered.

3 | RESULTS

Totally, 95 participations (50 in case and 45 in control group) enrolled. The clinical results showed that the most of case group were aged more than 35 years old with had higher parity and body mass index, but, no statistically significant difference showed up. On the other hand, although the lactate level was slightly higher in women with EP (Table 1), but, did not statistically differ between the two study groups.

Also, the logistic regression showed no relationship between the demographic variables (maternal age, parity, body mass index) and the lactate plasma level (Table 2).

4 | DISCUSSION

Our results showed that the plasma lactate level did not statistically differ between the two study groups, but clinically, the lactate level was slightly higher in women with EP. Also, there was no relationship between the demographic variables and the lactate plasma level.

TABLE 1 The variables distribution between the two study groups.

		Ectopic pregnancy ($n = 50$)	Normal pregnancy ($n = 45$)	p Value
Age *n* (%)	≥30	17 (41.5)	24 (58.5)	0.057[a]
	<30	33 (61.1)	21 (38.9)	
Parity *n* (%)	2≤	30 (46.2)	35 (53.8)	0.063[a]
	<2	20 (66.7)	10 (33.3)	
BMI *n* (%)	25≤	13 (26)	20 (44.4)	0.059[a]
	<25	37 (74)	25 (55.6)	
Lactate serum level (mg/dL) (M ± SD)		13.19 ± 5.50	11.42 ± 4.98	0.13[b]

[a]Chi-Square.

[b]Mann-Whitney test.

TABLE 2 The Logistic regression test among variables.

						95.0% C.I. for EXP(B)	
	B	S.E.	Wald	Sig.	Exp(B)	Lower	Upper
Age	−0.574	0.453	1.611	0.204	0.563	0.232	1.367
Body mass index	−0.824	0.465	3.139	0.076	0.438	0.176	1.092
Parity	−0.675	0.489	1.903	0.168	0.509	0.195	1.329
Lactate serum level	−0.073	0.043	2.969	0.085	0.929	0.855	1.010
Constant	1.864	0.722	6.661	0.010	6.451		

Although, the diagnosis of EP is based on a combination of β-hCG titration and vaginal ultrasound, but, these methods are still challenging for accurate and early diagnosis.[24] Currently, in more than 40% of cases, vaginal ultrasound cannot detect EP in the early stages. Also, a significant number of normal intrauterine pregnancies present low levels of β-hCG in the first days, and are not detectable in vaginal ultrasound, or expelled before the gestational sac formation.[24,25] On the other hand, in many EP cases, ultrasound cannot be an accurate diagnostic tool due to the report an unknown adnexal mass, para tubal cyst, corpus luteum, hydro salpinx, endometrioma, or even an intestinal loop![26] Also, the most common symptom of EP is vaginal bleeding and abdominal pain, which may be confused with abortion or normal intrauterine pregnancy.[27] Considering such challenges, it is important to identify new approaches to early diagnosis of EP and reduce the maternal mortality and morbidity. Such biomarkers should be easy to access, simple to analysis, inexpensive and with high sensitivity and specificity.

Here we assessed serum Lactate level in EP and the results showed no significant difference between case and control group. In a metabolomics survey on 2019, the diagnostic value of two metabolomics indicators (acetic acid and lactic acid) assessed during pregnancy and reported 92.3% and 96.6% sensitivity respectively, concluded that both metabolomics, especially lactic acid, can be an acceptable biomarker with high accuracy for diagnosing intrauterine pregnancy.[18] The other surveys on Lactic acid investigate its effect on endometrial-placenta layer and in the management of sepsis in pregnancy. Lactic acid drives from anaerobic metabolism. When a fertilized egg turns into a blastocyst, glucose is the main source of nutrition. During the first stages of embryo development, about 90% of glucose consumption is from the nonoxidative pathway and so it has been suggested that lactic acid creates an acidic environment in early pregnancy stages for embryo implantation.[19]

There are some studies reported a high amount of lactic acid secretion on utero-placental surface during fetal implantation with an unknown mechanism. On the other hand, the lactic acid role in tumor's local microenvironment and the similarities between tumoral cells and trophoblasts, it seems that lactic acid may have a similar role in the maternal-fetal interaction. Glycolysis process prevents the tumor progression through decreased oxygen status and increased lactic acid and improves the patient's survival. Therefore, it has been suggested that anaerobic or aerobic glycolysis may be an adaptive metabolic pathway in the placenta.[20,21]

In early pregnancy stages, lactic acid is effective in trophoblast invasion and angiogenesis. Also, it seems that lactic acid stimulates the decidua immune cells to tolerate fetal allogeneic immunity. However, researchers emphasize the need for more studies in this field.[22]

The inconsistency of our results with Turkogluld et al.[18] findings can be due to the slight changes of lactate serum level during pregnancy and other underlying factors such as parity and body mass index. Although the average plasma lactate level in our EP group was

higher than the control group (13.19 ± 5.50 vs. 11.42 ± 4.98), but, no significant statistical difference was observed between the two groups, while the clinical evidence shows that EP cases had a higher body mass index and parity. Maguire et al.[28] reported that lactate levels in early normal pregnancy (6–18 weeks) were directly related to body mass index and inversely related to gestational age.

Adjusting the underlying factors, we found no statistically significant difference between the two study groups. Because the release of lactate from adipose tissue occurs more in people with high BMI[29] and the incidence of EP increases in women over 35 years old, it seems that they must have a relationship with lactic acid titer and perhaps different results could be derived by matching the groups in terms of BMI and parity. However, these different findings and the lack of similar studies shows the importance of more surveys on this topic.

There is a theory reveals that during the early stages of pregnancy, placental blood supply is not stable and the oxygen concentration in the uterine lumen is relatively low (anoxic). Intravenous oxygen pressure increases from less than 20 mmHg in 8 weeks to more than 50 mmHg in 12 weeks and the lower oxygen concentration is associated with increasing glucose metabolism and high levels of lactic acid.[2] The other theory for higher level of lactic acid is the inflammatory condition during pregnancy such as more white blood cells, and higher ESR and CRP.

In a meta-analysis on 2019,[30] the normal range of lactic acid in healthy pregnant women reported as less than 2 mmHg during Labor and more than 4 mmHg in the rest of pregnancy period. Turkogluld et al.[18] declared that the combination of two indicators (lactic acid and acetic acid) can be a predictive clue, but due to the lack of similar studies regarding acetic acid in EP, this theory is with many challenges. Of the strengths of the present study is the exclusion of women with liver and kidney diseases which could affect the lactate level, as well as the sampling in the control group in 6–10 weeks of pregnancy to erase the effect of gestational age. Because it has been reported that the average lactate level in late pregnancy (36–42 weeks) is significantly higher than early pregnancy (6–18 weeks).[28] One of the limitations of the present study is the lack of smoking status and lack of matching in terms of demographic variables, while it has been reported that lactate level has a significant relationship with smoking in early pregnancy due to decreased the blood supply.[28]

5 | CONCLUSION

Our results showed that the plasma lactate level has no diagnostic power in EP, while the range of lactic acid in complicated pregnancies is unknown and the mechanism of lactic acid and the factors affecting it are unknown and need further studies.

AUTHOR CONTRIBUTIONS

Zahra Abbasi Ranjbar: Writing—original draft. **Seyedeh Hajar Sharami**: Writing—review & editing. **Fereshteh Fakor**: Conceptualization. **Forozan Milani**: Validation. **Roya Kabodmehri**: Supervision. **Zahra Haghparast**: Resources. **Seyedeh Fatemeh Dalil Heirati**: Project administration.

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CONFLICT OF INTEREST STATEMENT

The authors declare is no conflict of interest.

DATA AVAILABILITY STATEMENT

Supporting data are available in Reproductive Health Research Center, Department of Obstetrics & Gynecology, Al-Zahra Hospital, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.

ETHICS STATEMENT

The study was approved by the ethics committee of Guilan University of Medical Sciences (IR. GUMS. REC.1401.072). All stages of this research have been performed according to the Helsinki declaration. All procedures of the study were explained clearly to the participants who had the eligible inclusion criteria. Moreover, all participants voluntarily filled out the written informed consent form before they join the study and they were free to decide whether or not to attend or withdraw at any time and for any reason without changing the medical care.

TRANSPARENCY STATEMENT

The lead author Seyedeh Hajar Sharami affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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