

Activity of Lactate dehydrogenase in urine of spontaneous aborted women

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ABSTRACT

The present study was designed to estimate the activity of lactate dehydrogenase (LDH) in the urine of spontaneous aborted women and to evaluate the effect of some accompanied diseases such as; chronic hypertension and *Toxoplasma Gondii* infection on LDH activity . The comparison of 20 spontaneous aborted women was done with control groups of 20 healthy pregnant and 20 age-matched non-pregnant women. The results of this study showed a significant increase (p value=0.03) in urine LDH activity of aborted women compared with control groups. A significant increase (p=0.001) was recorded as well in LDH activity of aborted women with age of 30-45 years compared to 15-29 years group. The present study also revealed a significant increase (p=0.001) in LDH activity of aborted women accompanied with chronic hypertension and *Toxoplasma Gondii* infection.

فعالية انزيم اللاكتيت ديهيدروجينيز LDH في ادرار النساء المجهضات تلقائيا فراح غالي الصالحي* اسراء اسماعيل ياسين**تغريد عبدالحميد السعدون***

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الكلمات المفتاحية: انزيم اللاكتيت ديهيدروجينيز ، الاجهاض التلقائي ، جرثومة التوكسوبلازما

الخلاصة

صممت الدراسة الحالية لتقدير فعالية انزيم اللاكتيت ديهيدروجينيز (LDH) بادرار النساء ذوات الاجهاض التلقائي ، ومعرفة مدى تأثير بعض الامراض المصاحبة مثل: ارتفاع ضغط الدم المزمن و جرثومة التوكسوبلازما على فعالية LDH . شملت الدراسة (20) عينة ادرار من النساء المجهضات تلقائيا ومقارنتها مع (20) عينة ادرار لنساء حوامل يتمتعن بصحة جيدة و (20) عينة ادرار لنساء غير حوامل كمجموعتي سيطرة و باعمار متناظرة . اوضحت نتائج الدراسة ارتفاع فعالية LDH في ادرار النساء المجهضات مقارنة مع مجموعتي السيطرة ، وتبين وجود زيادة معنوية في فعالية LDH لدى النساء المجهضات ذوات الفئة العمرية (30-45)سنة مقارنة مع الفئة (15-29) سنة عند مستوى احتمالية $p=0.001$ ، كذلك ارتفع مستوى LDH عند النساء المصابات بارتفاع ضغط الدم المزمن و جرثومة التوكسوبلازما.

1. INTRODUCTION

Abortion is the termination of pregnancy by any means before the fetus is sufficiently developed to survive in life(1). Spontaneous abortion is defined as the natural termination of pregnancy prior the 20th week of gestation based upon the date of the first day of the last normal menses and the delivery of the fetus that weight less than 500gm, but in some European countries less than 1000gm(2).

The currently well-established causes together account for only about 40% of the abortion cases include chromosomal abnormalities , maternal diseases including poorly controlled diabetes mellitus , uncontrolled thyroid disease, severe systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS) ; poor maternal lifestyle habits (including alcohol consumption ,with a substantial proportion of cases (60%) classified as “unknown” or “unexplained”. The physiological hypoxia of the first trimester gestational sac

may protect the developing fetus against the deleterious and teratogenic effects of oxygen free radicals (OFRs) (3).

Lactate dehydrogenase (LDH) is an enzyme EC (1. 1.1.27) that functions in anaerobic glucose metabolism and glucose synthesis(4). LDH is present in a wide variety of organisms including plants and animals(5). Human beings have two identical subunits polypeptide chains with molecule mass of 30 - 35 kD for this enzyme: the H subunits, heart origin and M subunits originated from skeletal muscle. The functional enzyme is tetrameric, and many different combinations of the two subunits are possible, H₄, H₃M, H₂M₂, HM₃, and M₄ (6) .

LDH is mainly an intracellular enzyme. It is responsible for interconversion of pyruvate and lactate in anaerobic glycolysis. Its levels are several times greater inside the cells than in the plasma. So its levels are increased in the scenario of increased cell leakiness, hemolysis and cell death(7).

The present study was designed to estimate LDH activity in the urine of spontaneous aborted women and the effect of aborted women age. The study aimed also to evaluate the effect of some accompanied diseases such as; chronic hypertension and Toxoplasma Gondii infection on LDH activity in the urine of spontaneous aborted women .

2. MATERIALS AND METHODS

A cross sectional study was conducted taking women with spontaneous abortion & healthy pregnant women as cases and healthy non-pregnant women as controls. The study cases were selected from Tikrit Teaching Hospital. Case subjects were subdivided into three groups; Case group I- It included 20 diagnosed cases of spontaneous abortion in age group of 15-29 years. Case group II- It included 20 diagnosed cases of healthy pregnant women in age group of 30-45 years. Case group III(control)- It included 20 age matched healthy non- pregnant women without any major illness and who are not on any medication.

Activity of urine LDH was measured by a spectrophotometric assay by using analytical kits from Randox Co. In this method, the reaction was followed by measuring the rate of NADH consumption at 520 nm(8).

Values were calculated as mean±SD and the statistical analysis was done using SPSS 17.0 software. Student's unpaired t-test was used for comparison between two groups. The p-value of less than 0.05 was considered as statistically significant.

3. RESULTS AND DISCUSSION

In our study, it was seen that women with recurrent spontaneous abortion (RSA) (group I) were found to have significantly higher urine LDH levels compared to women with normal pregnancies (group II) and non-pregnant (group III) with $p \leq 0.03$, as shown in Table (1) and Fig.(1).

These finding was in accordance with study done by Qublan et al (9) and Kozic et al (10). They concluded that serum LDH can be a useful marker for prediction of adverse outcome of pregnancy in severe preeclampsia. Serum LDH has also found to be useful predictor for birth of small for gestational age infants in preeclamptic pregnancy(11). A group of researchers has noted significant usefulness of LDH levels in amniotic fluid at mid-trimester for prediction of fetal growth restriction (12).

It was found that LDH-A(4) isoenzyme is immunolocalized primarily in the fetal endothelial cells while LDH-B(4) isoenzyme is predominantly present in syncytiotrophoblasts. The LDH-A(4) isoenzyme activity increased approximately by 1.6 - fold

in preeclampsia when compared with normal pregnancy. This may also suggest that endothelial dysfunction present at uteroplacental vessels can lead to hypoperfusion to the growing fetus & may lead to elevation of LDH isoform (13).

Table(2) is a comparative table of age effect in aborted women and pregnant one, Comparatively with (15 - 29) years group in aborted women (131.7 ± 35.96) IU/L result presented significantly higher LDH activity in (30 - 45) years groups (199.7 ± 66.7) IU/L.

Oxygen toxicity is an inherent challenge to aerobic life and reactive oxygen species can modulate cellular functions and oxidative stress can impair the intracellular milieu resulting in diseased cells or endangered cell survival(11). The results of the present study showed that the significant increase in LDH activity in higher age group of aborted women might be due to increase oxidative stress in it(14).

Spontaneous abortion is accompanied by a significant disruption of the prooxidant and antioxidant balance. Oxidative stress may also have a role in patients with recurrent abortions with no known etiology(11). During pregnancy, there is an increased number of polymorphonuclear leucocytes (PMNL) that may result in increased generation of the superoxide ions(12).

In our study, we found significantly elevated levels of serum LDH in aborted women with chronic hypertension compared with controls (pregnant women), as shown in Table (3). These finding was in accordance with study done by Kozic et al(10). They concluded that serum LDH can be a useful marker for prediction of adverse outcome of pregnancy in severe preeclampsia.

It was found that LDH-A (4) isoenzyme is immunolocalized primarily in the fetal endothelial cells while LDH-B(4) isoenzyme is predominantly present in syncytiotrophoblasts. The LDH-A(4) isoenzyme activity increased approximately by 1.6-fold in preeclampsia when compared with normal pregnancy. This may also suggest that endothelial dysfunction present at uteroplacental vessels can lead to hypoperfusion to the growing fetus & may lead to elevation of LDH isoform(13).

Moreover the placenta is a major source of oxidative stress because of its enrichment with polyunsaturated fatty acid (PUFA)(15). suggested that the increase in the lipid peroxide levels was due to the increased prostaglandin synthesis in the placenta. Placental oxidative stress has been suggested to play a role in the pathogenesis of pre-eclampsia and fetal growth retardation(16).

Table (4) shows the effect of *Toxoplasma Gondii* infection on LDH activity in urine of spontaneous aborted women, in which there was a significant increase in LDH activity (163.995 ± 43.23) IU/L in aborted women compared with pregnant (95.755 ± 20.53) IU/L.

Toxoplasma Gondii is an obligatory intracellular protozoa parasite with a world-wide distribution which is capable of infecting all warm-blooded animals and is of both medical and veterinary importance. Toxoplasmosis is caused by a protozoal parasite that can be found in dried cat feces, contaminated soil, or contaminated water; and raw or undercooked meat containing infective tissue cysts. Although cats play a role in the epidemiology of the disease, there is no statistical correlation between toxoplasmosis infection and cat ownership. Toxoplasmosis can be transmitted to the fetus in utero through transplacental transmission(17).

Congenital infection caused by transplacental transmission can lead to a wide variety of manifestations in the fetus and infant including spontaneous abortion, still-birth, a newborn with classic signs of congenial toxoplasmosis such as hydrocephalus or microcephalus, cerebral calcifications and retinochoroiditis (18).

Two *Toxoplasma Gondii* genes were characterized that are differentially expressed during the parasite's life cycle. The genes named LDH1 and LDH2, respectively, encode polypeptides similar to the enzyme lactate dehydrogenase (LDH; EC 1.1.1.27) from a

variety of organisms. These results indicate that LDH expression is developmentally regulated in *T. Gondii*, and suggest a possible correlation between stage conversion and alteration in carbohydrate or energy metabolism in this parasite and its host(19).

REFERENCES

1. Raj, R. (2007). *recurrent miscarriage* , *Dewhursts textbook of gynaecology and obstetrics* ;7th edition : 100-105 .
2. Stirrat, G., *Lancet* , 336: 673, (1990).
3. Clifford, K.; Watson, H.; Grajewski, B.A. , *Hum. Reprod.* 9:1328, (1994).
4. Butova, O.A. & Masalov, S.V. , *Human Physiology*, 35(1): 127, (2009).
5. Kumar, V.; Roy, S.; Barman, D.; Paul, L. and Kumar, K., *Int. J. Agric.Sci.*, 3 (9): 005, (2013).
6. Jevery, M. B.; John, L.; Tymoczko, L. S. (2003). *Biochemistry*. 5th ed. W.H. Freeman and Company, New York, pp:274-275.
7. *Clinical enzymology and biomarkers*. In: Vasudevan D, Sreekumari S, Vaidyanathan K (eds). *Textbook of biochemistry*, 6th ed. Jaypee Brothers, New Delhi 2011, pp146-159.
8. Wroblewski, F. & LaDue, J.S. , *Proc Soc Exp Biol Med*,90:210,(1955)
9. Qublan, H.; Ammarin, V.; Bataineh, O.; Al-Shraideh , Z.; Tahat, Y.; Awamleh I et al., *Med Sci Monit*, 11(8): CR393 (2005).
10. Kozic, J.; Benton, S.; Hutcheson, J.; Payne, B.; Magee, L.; Dadelszen, P., *J Obstet Gynaecol Can* 33(10): 995,(2011).
11. Agarwal, A.; Gupta, S.; Sharma, R.K., *Reprod Biol Endocrinol* , 3:28, (2005).
12. Fait, V.; Sela, S.; Ophir, E.; et al., *J Soc Gynecol Investig* , 12:46,(2005).
13. Tsoi, S.; Zheng, J.; Xu, F.; Kay, H., *Placenta* , 22(4): 317, (2001).
14. Idonije, O.B.; Festus, O.; Okhiai, O. and Akpamu, U., *Research Journal of Obstetrics and Gynecology*, 4: 28, (2011).
15. Gitto, G.; Reiter, R.J.; Karbownik, M.; Tan, D.X.; Gitto, P.; Barberi S. and Barberi, I. *Biol. Neonate*, 81: 146, (2002).
16. Takagi, Y.; Nikaido, T.; Toki, T.; Kita N.; Kanai M. et al., *Virchows Arch.*, 444: 49, (2004).
17. Pinard, J.A.; Leslie, N.S.; Irvine, P.J., *J-Midwifery-Womens-Health*, 48(5): 308; quiz 386, (2003).
18. Goldenberg, R.L.; Thompson, C., *Am J Obstet Gynecol*, 189:861, (2003).
19. Yang, S. and Parmley, S.F., *Gene* , 184(1): 1,(1997).

Table (1) :LDH activity in urine of spontaneous aborted women

Groups	No.	Mean \pm SD LDH activity (IU/L)
Aborted women	20	164.86 \pm 56.9
Pregnant women (control)	20	95.755 \pm 20.53
non-pregnant	20	72.72 \pm 34.03
P value	-	0.03

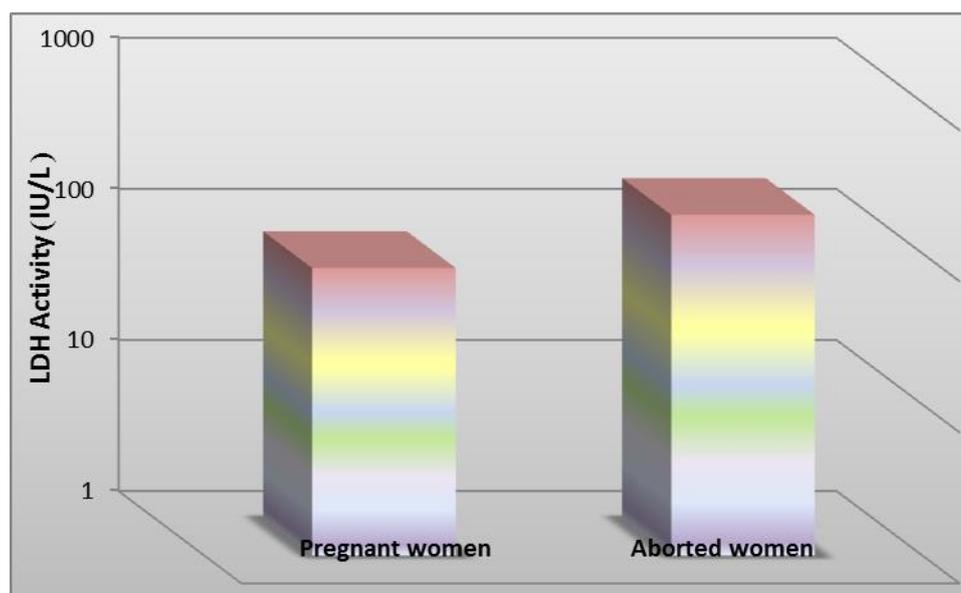
**Figure (1): Elevated LDH activity in urine of spontaneous aborted women**

Table (2) :LDH activity in urine of aborted women according to age groups

Age (year)	((pregnant women))		((aborted women))		P value
	Mean \pm SD	LDH activity (IU/L)	Mean \pm SD	LDH activity (IU/L)	
15 – 29	82.764 \pm 21.96	(No. = 12)	131.678 \pm 35.9	(No. = 12)	0.001
30 - 45	95.463 \pm 13.06	(No. = 8)	199.663 \pm 66.7	(No. = 8)	0.001

Table (3) :Activity of serum LDH in aborted women with chronic hypertension

Case	No.	Mean \pm SD	LDH activity (IU/L)	P value
Pregnant women (control)	20	95.755 \pm 20.53		0.001
Aborted women (accompanied with chronic hypertension)	10	145.46 \pm 48.17		

Table (4): The effect of *Toxoplasma Gondii* infection on LDH activity in urine of spontaneous aborted women.

Case	No.	Mean \pm SD LDH activity (IU/L)	P value
Pregnant women (control)	20	95.75 \pm 20.53	0.001
Aborted women (accompanied with <i>Toxoplasma Gondii</i> infection)	10	163.995 \pm 43.23	