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Significance of weakly positive urine pregnancy tests

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Abstract: *Objective:* The objective of this study was to determine the pregnancy outcome of weakly positive urine pregnancy tests in 207 women by transvaginal sonograghy (TVS). *Background:* Urine pregnancy test (UPT) also called Home pregnancy test (HPT) currently make up the fastest growing segment of home diagnostic testing market. These tests give a significant results as a weakly positive pregnancy test (hCG 25-50IU/L) and a positive pregnancy test (hCG >50IU/L), but both the results are reported as a positive pregnancy test by a non- professional. Human gonadotropin (hCG) levels indicate the health of the trophoblastic tissue, low level of βhCG is associated with poor decidual reaction. A weakly positive result indicates low hCG level and requires further evaluation. *Method:* Women visiting Antenatal Clinic for confirmation of pregnancy with weakly positive UPT were advised repeat UPT after 48hours on morning urine sample, repeat UPT with weakly positive results were advised TVS to known the pregnancy outcome. *Result:* TVS of these weakly positive results showed early pregnancy failure (EPF) in 138(66.6%), viable pregnancy 58 (28%) and no pregnancy in 11(5.3%). *Conclusion:* Thus these kits require a manufactures instruction to distinguish positive and weakly positive results and an advice for further evaluation by an expert to known the pregnancy outcome and to prevent the complications of interference in non- pregnant status and continuation of EPF.
Keywords: weakly positive urine pregnancy test, transvaginal sonography, first trimester.

Introduction

Cessation of menstruation in regularly menstrual-ting women is an anxious situation both in infertility and unwanted pregnancy. Most of the women use HPT kit to determine their pregnancy status before seeking professional health care. Sensitivities of 10-50IU/L became possible with the availability of monoclonal antibodies and refined techniques capable of giving positive result for pregnancy at the time of the first missed period. The test utilizes combination of antibodies including mouse monoclonal anti-hCG antibodies and goat polyclonal anti-hCG antibodies to selectively detect elevated levels of hCG in urine. The assay is conducted by adding 2 drops of urine to the specimen well of the test device and observe the formation of colored lines. The urine migrates via capillary action along the membrane to react with the colored conjugates. Positive result form a colored line at the test line region of the membrane by reacting with the specific colored antibody conjugates. Low level of hCG in urine give weakly positive results. Mostly the manufactures of these kits do not quote the sensitivity of these kits and quote both positive and weakly positive result as positive for pregnancy. Some devices give range of time within which the result may be read. The result is more sensitive if read after a longer interval but it is not known what time each manufacturer used to determine the sensitivity of its device.

Weakly positive results may be due to poor sensitivity of the device, hypersensitivity of the device, the hook effect (excessive hCG as in molar or multiple gestation would swamp the binding sites of both the antibodies) and cross reaction with lutenizing hormone (LH). Low levels of hCG can occur in apparently healthy, non pregnant subjects [1-2]. Peri and post menopausal urine may elicit a weak positive result due to low hCG levels unrelated to pregnancy[2]. hCG values double approximately every 48 hours in normal pregnancy (at 4^{th} week gestation the mean β subunit doubling times are approximately 2.2days and fall to 3.5 days by 9th week gestation) [3], thus patients with low level of hCG should be sampled and retested after 48 hours. Serial quantitative hCG in serum is advised in monitoring early pregnancy

complications that is yet to be documented as viable or intrauterine [4]. Weakly positive or positive results is a possibility because of heterophilic antibodies, non specific protein binding, altered forms of hCG, hCG like substances, trophoblastic and non- trophoblastic neoplasms and also in patients who have received mouse monoclonal antibodies for diagnosis or therapy, may contain anti-mouse antibodies (HAMA) which cross reacts with hCG [5-6].

TVS changed the trend in the diagnosis of early pregnancy viability and location. TVS is commonly performed in the 1st trimester of pregnancy to confirm pregnancy location, viability or gestational age (Bigrigg and Read, 1991). TVS with transducer of 5-10 MHz and higher resolution has revolutionized the understanding of pathophysiology and the management of early pregnancy complications.

Sonographic predictors of poor pregnancy outcome are:

1. Gestational sac (GS) appears by 31-35 days gestational age, double decidual sign with mean sac diameter (MSD) of 10mm at 40day gestational age (GA), Mean sac growth is 1.13mm/day, it is abnormal if mean sac growth is <0.70mm/day [7],
2. Distorted sac shape, a thin (<2mm) weakly echogenic or irregular choriodecidual reaction indicates EPF [8]
3. When GA is 5.5-9week the mGS size is normally at least 5mm greater than the crown rump length (CRL), if the difference is less than 5mm spontaneous abortion is the outcome [9].
4. Absence of Yolk Sac (YS) with a MSD of 13mm, large YS of >6mm, calcified or echogenic YS or double appearance of YS indicates poor pregnancy outcome [10]
5. Absence of visible embryo, YS with MSD of 20mm is anembryonic pregnancy [11]
6. Absence of cardiac motion with embryo measuring 5mm is missed abortion [11]
7. Normally amnion is thin and not visualized, if easily seen it is thick and abnormal, enlarged amnion is also associated with embryonal death [12-13]
8. Adnexal mass with absence of intrauterine gestational sac indicates ectopic pregnancy [13]

Material and Methods

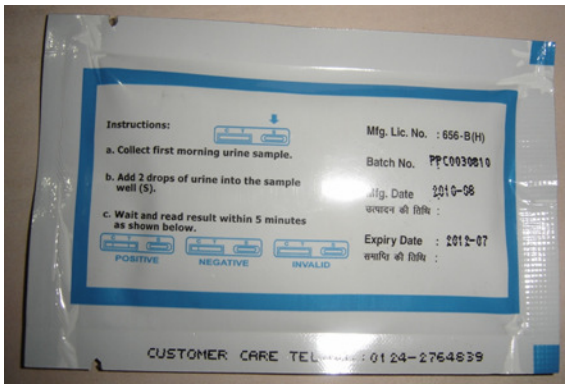
This was a prospective observational cohort study of women attending antenatal clinic of Al- Ameen Women and Children Hospital for confirmation of pregnancy from April 2009 to September 2011, with the permission of hospital ethical committee 207 women included in this study.

Inclusion criteria: Women of reproductive age (16-43years), amenorrhea (37-55days) with or without symptoms/ signs of pregnancy, with known LMP and regular past menstrual cycles. Exclusion criteria: Women on exogenous hCG, irregular menstrual cycle, recently on oral contraceptive pills, lactating, amenorrhea with pain abdomen and bleeding per vagina, not sure about LMP and women who refused TVS.

Per speculum and pelvic examination were done to note the signs of pregnancy, size of the uterus and the adnexa. Urine pregnancy test done by P-test, rapid one step hCG, manufactured and marketed by Hindustan Latex limited. Women who tested weakly positive, were advised to undergo repeat UPT after 48 hours with their morning urine sample, women who tested weakly positive in the repeat UPT were included in this study. TVS with Aloka prosound2 vaginal probe of 5MHz, done immediately and repeat TVS after 10 days to know their pregnancy outcome. They were advised to report immediately if there was h/o pain abdomen or bleeding per vagina.

Fig-1: (a). kit showing weakly positive UPT
(b). kit showing positive UPT

Fig-2: Showing instructions on the kit



Results

The prevalence of weakly positive pregnancy test was 0.8% in laboratory samples. Of 340 repeat UPT on morning urine sample after 48hour, 250 (73%) showed weakly positive UPT, 43 refused TVS, thus 207 included in the study. Acceptance of TVS was 82.8%. Mean age was 30±7 years, mean gravidity was 3 and mean amenorrhea duration was 45±3days.

Table-1: The TVS finding of the 1st Scan and the follow up 2nd scan after 10days

TVS FINDING	1ST SCAN n=207	2nd scan done after 10 days
Viable pregnancy corresponding to the period of amenorrhea	40	33(15.9%) continued as viable pregnancy, EPF in 7(3.3%) 2 had spontaneous abortion and 5 had missed abortion.
Fetal echo of >5-6mm CRL with no cardiac activity (missed abortion)	64 (30.9%)	
Pregnancy of uncertain viability GS <20mm with no yolk sac, fetal pole	90	67(32%) were anembryonic (5 reported with bleeding pv within 10day 23(11.1%) were viable pregnancy
Pregnancy of unknown location (no sign of intrauterine or extra uterine pregnancy)	12	11(5.3%) were non-pregnant 1(0.4%) showed viable intrauterine pregnancy
Multiple pregnancy with twin viable GS of 9weeks	1	1(0.4%)

The final outcome of the weakly positive pregnancy test were, EPF in 66.6% (missed abortion in 71 (34%) and anembryonic pregnancy in 67 (32%) women), Viable pregnancy in 55(28%) women, which included 1 twin gestation (probably due to the hook effect) and another with pregnancy of unknown location progressed to viable intrauterine pregnancy. 11 (5.3%) were non pregnant out of whom 9 women belonged to the age group of 39-43 years (probably due to pituitary hCG or cross reaction with LH).

Table-2: The final outcome after second TVS were

Early pregnancy failure (anembryonic=67 and missed=71)	138(66.6%)
Viable pregnancy (singleton=57,twin=1)	58(28%)
Non pregnant	11(5.3%)

Table-3: Women with pregnancy symptoms

Viable pregnancy (n=58)	40(68.9%)
Non- viable pregnancy (n=138)	100(72%)
Non pregnant (n=11)	5(45%)

Pregnancy symptoms included morning sickness, breast tenderness and fullness, urinary frequency and fatigue. Irrespective of viability and non-pregnant status 155(74.5%) showed pregnancy symptoms, thus symptoms of pregnancy alone is not significant in the diagnosis of pregnancy or viability of pregnancy.

Table-4: Signs of pregnancy (Chadwick's and Hegar's sign)

Viable pregnancy n=58	45(77%)
Non- viable pregnancy n=138	70(50.7%)
Non pregnant n=11	1(9%)

77% of viable pregnancy showed positive signs of pregnancy and 50.7% nonviable pregnancy also showed pregnancy signs, but only 1 (9%) non-pregnant woman showed Chadwick's signs indicating pregnancy sign is important to diagnose pregnancy but not the viability.

Discussion

Ekawong P et al reported incidence of 0.57% weakly positive UPT [14]. Our prevalence was 0.8% in the laboratory urine sample. The optimal timing of an USG to assess the location and viability of an early pregnancy is 49 days [15], mean gestational age was 45±3days in our study. The chances of diagnosing an ectopic pregnancy is three times higher in women with pregnancy associated with symptoms of pain and bleeding than in women without these symptoms [15]. Our study did not include women with pain or bleeding, and no women had ectopic pregnancy. The diagnosis of normal pregnancy and abnormal pregnancy requires a multifaceted approach like history, physical examination, laboratory test and ultrasonography.

Pregnancy is suspected whenever a women of childbearing year who has had regular menstrual cycle notices abrupt cessation of her menses. Zabin L S et al stated that delayed menstrual cycle had a sensitivity of 68% and specificity of 40% for pregnancy [16]. J.R Coll et al noted that the symptom of amenorrhea was 63% sensitive and 59.3% specific for pregnancy and morning sickness had sensitivity of 39% and specificity of 86% for pregnancy [17]. Thus amenorrhea and symptoms of pregnancy are not conclusive of pregnancy. Chadwick noted that sensitivity of Chadwick sign was 51% and specificity of 98% for pregnancy diagnosis [18] and MacDonald E in his study on pregnant women found to have Hegar sign in 94% and Chadwick sign in 61% [19]. Our study showed 77% of viable and 50.7% of nonviable pregnancy with positive pregnancy signs thus not diagnostic of viability of pregnancy. When diagnosing pregnancy, the patient or the clinician should not rely on symptoms and signs of pregnancy or a HPT, a laboratory urine pregnancy test should be requested because accuracy depends on several factors like reading the instructions carefully and the number of days beyond the missed period the test is done [20].Chan. Y F et al noted the specificity of a weakly positive urine pregnancy test in predicting poor pregnancy outcome was 98.8% and the sensitivity was 28.6%. [21]. In our study 66.6% had EPF and 5.3% were non pregnant. One third of women who think they are pregnant have an HPT [22] and 28% of adolescents use HPT prior to their visit to consultant [17]. Thus weakly positive results may affect the feasibility and safety of pregnancy termination and unnecessary continuation of EPF and interferences in non-pregnant status.

Conclusion

It is unlikely that we can prevent teenagers and adults from using HPT kit. Alternatively suggestions to encourage manufacturer to label kits with their sensitivity, clear instructions how to use and also the time within to be read, pictorial demonstration of results and warning suggestion that users should consult their doctors irrespective of negative, weakly positive or positive result is mandatory. Whenever a women presents with delayed menstrual cycle with a HPT kit, it should always be retested by a professional operator with experience of pregnancy testing and TVS advised to known the pregnancy outcome and non-pregnant status.

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References

1. Alfthan H, Haglund C, Dabek J, Stenman UH. Concentrations of Human Choriogonadotropin, its β-subunit, and the core fragment of the β-subunit in serum and urine of men and non-pregnant women. *Clin Chem* 1992; 38: 1981-87.
2. Borkowski A, Muquardt C. Human chorionic gonadotrophin in the plasma of normal, non pregnant subjects. *N Eng J Med* 1979; 301:298-302.
3. Mchesney R. Wilcox AJ, O'Connor JF. Intact HCG, free HCG beta subunit and HCG beta core

fragment longitudinal patterns in urine during early pregnancy. *Hum Reprod* 2005; 20(4): 928-35.
4. Silva C, Sammel MD, Zhon L, Human Chorionic gonadotrophin profile for women with ectopic pregnancy. *Obstet Gynecol* 2006; 107(3): 605-10.
5. Boscato CM, Stuart MC. Heterophilic antibodies; A problem for all immunoassays. *Clin Chem.* 1988; 34:27-33.
6. Schroff RW, Foon KA, Beatty SM, Oldham RK, Morgan Jr AC. Human anti-urine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 1985; 45:879-85.
7. Nyberg DA, Mack LA, Laing FC, Patten RM. Distinguishing normal from abnormal gestational sac growth in early pregnancy. *J Ultrasound Med* 1987;6: 23-27.
8. Bajo J, Moreno-Calvo FJ, Martinez-Cortis L, Haya FT, Raymond J. Is trophoblastic thickness at the embryonic implantation site a new sign of negative evolution in first trimester pregnancy? *Hum Reprod* 2004:15:1629-1631.
9. Benacerraf BR. Small sac size in the first trimester, a predictor of poor fetal outcome. *Radiology* 1991; 178: 375-77.
10. Kurjas, Kuperic S. Parallel Doppler assessment of yolk sac measurement with vaginal sonography in first trimester in the prediction of pregnancy outcome. *Acta Obstet Gynecol. Scand,* 1997; 76: 969-72.
11. Jauniaux E, Johns J, Burton G .The role of ultrasound imaging in diagnosing and investigation of early pregnancy failure. *Ultrasound Obstet Gynecol,* 2005; 25: 613-624.
12. Harrow MM, Enlarged amniotic cavity, a new sonographic sign of early embryonic death. *AJR* 1992; 158.
13. Perriera L, Reeves M F. Ultrasonographic criteria for diagnosis of early pregnancy failure and ectopic pregnancy. *Semin Reprod Med* 2008; 26(5):373-82.
14. Ekawong P, Miwanitkit V. Weakly positive urine pregnancy diagnostic test rate. *Chula Med J* 2006; 50(12): 863-7.
15. Bottomley C, Van Belle V, Mukri F, Kirk E, Huffel V, Timmerman D, Bourne T. The optimal timing of an ultrasound scan to assess the location and viability of an early pregnancy. *Hum Rep* 2009; 24(8):1811-17.
16. Zabin LS, Emerson MR, Ringers. PA, Sedivy V. Adolescents with negative pregnancy test results an accessible at risk group. *JAMA* 1996; 275: 113-117.
17. Coll JR. Early diagnosis of pregnancy in general practice. *Gen Prac,* 1977; 27: 335-338.
18. Chadwick JR. Value of the bluish coloration of vaginal entrance a sign of pregnancy. *Trans Am Gynecol,* 1887; 11: 399-418.
19. MacDonald E. The diagnosis of early pregnancy with report of 100 cases and special reference to the sign of flexibility of the isthmus of the uterus. *Am J Obstet Dis Women Child.* 1908; 57: 323- 346.
20. Lori. A. Bastian, MD, MPH, Joanne. T, Piscitelli. MD. Is the patient pregnant? Can you reliably rule in or rule out early pregnancy by clinical examination? *JAMA,* 1997; 278(7); 586-591.
21. Chan YF, So WW, Yeung WS, Lau EY. Ho PC. The value of a single sensitive urine pregnancy test in prediction of pregnancy outcome. *Asia Oceania J Obstet Gynecol,* 1994; 20(4): 401-5.
22. Lori Bastian MD, MPH, Kavitha Nanda. M D, MHS, Vic Hasselblad. PhD, David L, Simol MD, MHS. Diagnosis Efficiency of Home Pregnancy Test Kits. *Arch fam Med.*1998; 7: 465-469.

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