

## Dentistry

**KEYWORDS:** Human chorionic gonadotropin , pregnancy, saliva, salivary diagnostics.

**SALIVARY HUMAN CHORIONIC GONADOTROPIN AS A NOVEL BIOMARKER FOR EARLY DETECTION OF PREGNANCY – AN EVOLVING CONCEPT**



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**ABSTRACT**

**Introduction:** Saliva is a clinically important biologic fluid. The present study aims at determining the role of saliva in detection of pregnancy using home-based pregnancy detection kits to provide an alternative biofluid that is more user friendly, acceptable, non-invasive, rapid and easy for home use.

**Materials and method:** The study was conducted among 100 women visiting a gynecology clinic for confirmation of pregnancy with a history of a missed menstrual cycle not more than 4 weeks. The test will be performed using pregnancy detection kit with 20 mIU specification for estimation of salivary hCG. Routine tests were prescribed to the patients such as laboratory based urine hCG and/ultrasound examination was used for confirmation of pregnancy status & correlation with the saliva based result.

**Results:** About 84% participants with a confirmed pregnancy showed a positive result, of which 62% showed a dark band & a light band was noted in 22% participants. A negative result was noted in 16% participants & were positive for urine test result.

**Conclusion:** The result of this study showed that traditionally used biofluid can be replaced with more user friendly & acceptable biofluids. i.e., saliva as a biomarker for detection of pregnancy. In this study urinary hCG based kit was used but it opens new avenues towards development of new Salivary based diagnostic kits for detection of pregnancy.

**INTRODUCTION:**

The evolution of salivary diagnosis has reached a level towards the goal of using saliva as a powerful fluid for early detection of pregnancy and also it is used for first line diagnosis for life threatening diseases such as cancer, metabolic disorders, infections etc. 1. The collection of saliva is simple, painless, cheap and safe procedure both for patient and medical staff. An additional advantage of saliva is the fact that it may be retrieved several times

a day, which make repeat analysis much easier. Furthermore, saliva has very high durability. Although 94-99% of saliva content is water, saliva also contains numerous cellular elements and many organic and inorganic substances, including most biological markers present in blood and urine that may be used in the early detection and monitoring of many dental and general disease<sup>2</sup>.

Human oral fluid that is saliva contains numerous molecules that play a variety of roles. Among them the presence of human chorionic gonadotropin hormone which serves as a biomarker in detection of early pregnancy. Pregnancy test for human chorionic gonadotropin are used widely in hospital and home settings<sup>7</sup>.

In human chorionic gonadotropin hormone there are two subunits present: they are Alpha and Beta subunits. In that beta human chorionic gonadotropin in plasma and urine are currently used in the detection of pregnancy. Beta human chorionic gonadotropin levels in saliva are usually detectable at about 3-4 weeks of pregnancy and continue to increase throughout the pregnancy<sup>4</sup>. However only a few studies have been carried out to evaluate the role of saliva as a non-invasive, rapid and more acceptable biofluid for pregnancy detection. With this objective, the present study was carried out to determine the role of saliva in the detection of pregnancy using home based pregnancy detection kits.

**METHODOLOGY**

The study was conducted among 100 women visiting a gynecology hospital for confirmation of pregnancy with a history of a missed menstrual cycle not more than 4 weeks. Prior to the conduction of study ethical approval was done, then informed consent was obtained after thoroughly explaining them about the procedure. The participants were in the age range of 22-30 years. The participants were divided into three groups. Group - I participants had a history of missed period of less than or equal to 10 days, Group - II between 11 to 15 days and Group - III more than 15 and 30 days. After taking informed consent saliva was collected in a sterile container through spitting method. A home-based pregnancy detection kit meant for urinary hCG with 20 mIU specification was used in the study for estimation of salivary hCG. Routine test that was prescribed for the patient such as laboratory-based urine hCG and /or ultrasound examination was

used for confirmation of pregnancy and for correlation with the saliva-based result [Figure 1].



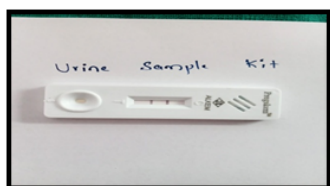
**Figure: 1** Saliva sample collection containers and method of testing.

**STATISTICAL ANALYSIS**

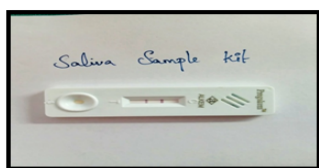
Results were expressed as Mean standard deviation , range values and number of percentages.Kruskal –Wallis ANOVA was used for multiple group comparisons followed by wilcoxon's rank sum test.

**RESULTS**

All the participants evaluated were in the age group of 22-30years .About 73% participants had a history of missed period of less than or equal to 10 days, 79% between 11 and 15 days and 19% more than 15 and 30 days [Table - 1]. About 84% participants with a confirmed pregnancy showed a positive result, of which 62% showed a dark band and a light band was noted in 22% participants [Figure 2, 3 & 4]. A negative result was noted in 16% participants and were positive for urine test results. Hence, in the present study, we observed 84% accuracy and 16% false – negative results [Chart – 1].



**Figure: 2** Test kit showing positive result with urine sample.



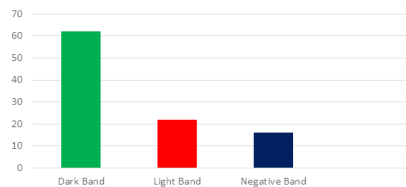
**Figure: 3** - Test kit showing positive result with saliva sample (dark band).



**Figure: 4**-Test kit showing positive result with saliva sample (light band)

**Table 1 : Showing results of the salivary hCG test**

Saliva Results	Results of the Salivary HCG test			
	Days after missed periods			
	≤10 days	11-15 days	≥15 days	Total
Dark Band	15	40	02	57
Light Band	11	02	04	17
Negative	14	12	-	26
Total	40	54	06	100



**Chart 1 :** Gestational age when the salivary hCG test was performed

early detection of disease as it contains specific soluble biological markers. These multiple biomarkers make saliva useful for multiplexed assays that are being developed as point-of-care devices , rapid test or in more standardized formats for centralized clinical laboratory operations <sup>3</sup>. Hormonal analysis of saliva is not influenced by changes in concentration of binding globulins as the free concentration of the hormones is measured <sup>5</sup>.

Human chorionic gonadotropin hormone plays an important role in detection of early pregnancy. It is a hormone produced primarily by syncytiotrophoblastic cells of the placenta during pregnancy . The hormone stimulates the corpus luteum to produce progesterone to maintain the pregnancy <sup>6</sup>.

Certain malignancies can also produce either human chorionic gonadotropin or human chorionic gonadotropin –related hormone. Such as trophoblastic cancer [ hydatidiform mole, choriocarcinoma, and germ cell tumors ] are associated with high serum level of human chorionic gonadotropin –related molecules <sup>6</sup>. The normal values of human chorionic gonadotropin hormone in pregnant woman is greater than 25 mIU/mL, Non –pregnant woman- less than 5 mIU/mL, Post menopausal woman is less than 9.5 mIU/mL and men – less than 2 mIU/mL.

Human chorionic gonadotropin is basically a glycoprotein composed of two subunits , alpha and beta hCG subunits. It contains 237 amino acids in that alpha –hCG subunit is 92 amino acids and beta-hCG subunit is 145 amino acids. Its molecular mass is 36.7 kDa, approximately 14.5 kDa alpha-hCG subunit and 22.2 kDa beta-hCG subunit <sup>4</sup>. It is produced in 3-4 weeks after conception. It is primarily catabolized by liver ,although about 20% is excreted in urine <sup>6</sup>. The beta hCG subunits is degraded in kidney to make a core fragment which is measured by urine hCG test. Three major forms of hCG are produced by humans .These includes regular hCG, Hyperglycosylated hCG, and free beta subunit of Hcg <sup>4</sup>.

Study by Liu et.al on quantification of steroid hormones in human saliva, stated that steroid hormones follow the same metabolic pathway and therefore have an unequivocal degree of correlation with respect to their concentrations <sup>8</sup>.

The study on proteomic analysis of women saliva during fertile phase of menstrual cycle by Saibaba et.al. in 2016 , conclude that Cystatin –S is highly expressed protein during ovulation <sup>8</sup>.

Study by Julia Stern et .al.in 2022 on testosterone levels of saliva and hair samples were almost equally stable across the ovulatory cycle <sup>9</sup>.

Detection of pregnancy is based on the measurement of hCG and its variants. In a normal menstrual cycle , ovulation occurs on an

average of 14 days before the next anticipated menses and the corpus luteum then start to develop<sup>8</sup>. If the oocyte is fertilized, implantation takes place approximately during the next 7 days, resulting in an increase in the concentration of human chorionic gonadotropin and its variant. Urinary hCG level of 25 U/mL is generally considered positive urine pregnancy test result. Based on the results of the previous studies, we intended at evaluating if saliva could be used as a potential biomarker for detection of pregnancy.

In the present study, home-based pregnancy detection kit meant for urinary hCG detection with 20 mIU specification was used for estimation of salivary hCG. Human oral fluid that is saliva is considered as an alternative matrix for monitoring of biochemical parameters. A graphene-based chemiluminescence resonance energy transfer immunoassay for the detection of hCG in the serum and saliva samples had been utilized. The result of their study indicated that hCG level could be detected in the saliva samples in the concentration ranging from 0.3 mIU/mL to 0.8 mIU/mL.

In the present study, although we used home-based pregnancy detection kit meant for urinary hCG detection. Estimation using these kits showed a 84% accuracy and 16% false-negative. In this study we find 73% participants had a history of missed period of less than or equal to 10 days, 79% between 11 to 15 days and 19% greater than 15-30 days. Hence the test was able to detect pregnancy status in most women at a gestational age of 2-3 weeks or lesser.

### CONCLUSION

The result of this study showed that traditionally used bio fluid can be replaced with more user friendly and acceptable biofluid that is saliva as a biomarker for detection of pregnancy.

In this study, urinary hCG based kit was used but it opens new avenues towards the development of new saliva based diagnostic kits for detection of pregnancy. With the findings obtained from our study we could positively confirm that salivary Hcg can be used as a potential, user friendly, more acceptable home based biomarker for early detection of pregnancy.

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