Ovulation detection through salivary levels of sialic acid and glycosaminoglycans

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ABSTRACT
Background: One in ten couples of reproductive age encounter some level of infertility. Identification of the period of ovulation in humans is critical in the treatment of infertility. Success in in vitro fertilization and embryo transfer has been associated with the exact time of ovulation. Saliva is a unique diagnostic fluid, the composition of which immediately reflects the sympathetic nervous system, parasympathetic nervous system, hypothalamic-pituitary-adrenal axis and immune system response to stress. The study aims at evaluating the changes in salivary sialic acid and Glycosaminoglycans in the regular menstrual cycle. Thus, the presence of these carbohydrates in the ovulatory saliva makes the possibility to develop a biomarker for the detection of ovulation by noninvasive methods.

Subjects, materials and methods: Randomly, seventy five volunteer females were recruited and divided into 5 groups each contains 15 subjects as follow: Nine years old females and postmenopausal females as control groups, pre-ovulatory period, ovulatory period and post-ovulatory period females as experimental groups. Each female, of the experimental groups, underwent sonographic examination to estimate her period regarding ovulation. Unstimulated whole saliva was collected using the spitting method. Colorimetric procedure was used for total sialic acid determination and for Glycosaminoglycans quantitative determination, the method of ELISA was used.

Results: The concentration of sialic acid was significantly decreased in saliva of females in the ovulatory phase of the menstrual cycle; whereas, a significant increase in salivary sialic acid concentration was in the post-ovulatory phase. Glycosaminoglycan concentration showed a gradual increase from the pre-ovulatory phase then ovulatory to reach its maximum in the post-ovulatory phase with a significant difference between the pre-ovulatory and post-ovulatory phases. A significant correlation was not found between sialic acid and Glycosaminoglycans in different study groups.

Conclusions: On the basis of the results arrived at, the study concluded that there are remarkable cyclic variations in sialic acid and glycosaminoglycans during the menstrual cycle but in conclusion, glycosaminoglycans and sialic acid salivary levels cannot be used for the precise prediction of ovulation.

Keywords: Ovulation, saliva, sialic acid, glycosaminoglycans.

INTRODUCTION
The cyclic physiologic changes are mainly brought about by the ovarian hormones estrogen and progesterone, the levels of which show variation during the menstrual cycle. Identification of the period of ovulation in humans is critical in the treatment of infertility. Success in in vitro fertilization and embryo transfer has been associated with the exact time of ovulation. In the recent years, attention has been paid to the noninvasive method in ovulation detection (1). Saliva is a unique diagnostic fluid, the composition of which immediately reflects the sympathetic nervous system, parasympathetic nervous system, hypothalamic-pituitary-adrenal axis and immune system response to stress (2).

Recent reports shows that the saliva is a very good source of both hormones and biochemicals and that their levels change in accordance with the menstrual cycle (1). Carbohydrates are the major diet for mammalian species. The nature of the feeding habit would have a major impact on the excretion of biomolecules. This may be the reason for a considerable release of carbohydrates in the saliva (3,4). Most of the salivary proteins are glycoproteins. Sialic acid (SA) is one of the terminal sugars of salivary glycoproteins. It is an important structural component of salivary glycoproteins, enhancing bacterial aggregation as well as participating in the formation of the acquired pellicle and dental plaque (5). A previous study suggests that bovine submaxillary mucin has hydroxyl radical scavenging ability and the SA in mucin is an essential moiety to scavenge hydroxyl radicals and mucin synthesis is induced by oxidative stress (6).

Proteoglycans are macromolecular components of the extracellular matrix that play various roles in normal cell physiology and in pathologic states (7). Modulation of proteoglycan turnover by follicular stimulating hormone (FSH) and luteinizing hormone (LH) is mostly related to the ovulatory process (8). A recent study suggests changes in salivary glycosaminoglycan (GAGs) and sialic acid.
acid that are parallel to the normal increases in serum estrogen levels that occur in normal menstrual cycle \(^{(1)}\).

**MATERIALS AND METHODS**

This is a cross-sectional study in which seventy five volunteer females were recruited and divided into 5 groups; each contains 15 subjects as follow:

- **Group A:** Eight to nine years old females represent the pre-pubertal period. (Control group)
- **Group B:** Reproductive age females in the pre-ovulatory period. (Control group)
- **Group C:** Reproductive age females in the ovulatory period. (Experimental group)
- **Group D:** Reproductive age females in the post-ovulatory period. (Experimental group)
- **Group E:** Postmenopausal females (10-20 years after menopause). (Control group)

Each female, of the experimental groups, underwent sonographic examination first to estimate her period regarding ovulation.

**RESULTS**

Saliva sialic acid and GAGs concentrations were almost constant in different age groups and not related to age. In the experimental groups, salivary sialic acid level decreased in the pre-ovulatory phase compared to control groups. Nadir level was obvious in the ovulatory phase, and then a sudden rise was found in the postovulatory phase making the highest concentration. Fig 1.

A high statistically significant difference between SA conc. in the ovulatory phase and the post-ovulatory phase \((p= 0.001)\). Another high significant difference was found between the ovulatory phase and the prepubertal group \((p=0.001)\). A higher significant difference was found between ovulatory and postmenopausal group \((p=0.001)\). The only non-significant value was between ovulatory and pre-ovulatory periods \((p=0.973)\).

Salivary GAGs in normal young women presented a biphasic pattern, with higher concentration values during the second half of the menstrual cycle. In the experimental groups, the lowest salivary GAGs level was in the pre-ovulatory phase compared to control groups followed by a gradual increase in the ovulatory phase and the peak level was in the post-ovulatory phase as shown in fig 2. A statistically significant difference was found between the pre-ovulatory phase GAGs level and the post-ovulatory phase level \((p=0.013)\). Also the levels showed a high significant difference between the pre-ovulatory phase and the postmenopausal group \((p=0.0001)\). No significant differences were found among the other groups.

**DISCUSSION**

**Sialic acid**

According to this study, SA level in old age group (above 45) were almost similar to that of children (8-9 years old) and are also not far away from its level in young adult except for its level in the post-ovulatory phase. This means that salivary sialic acid concentrations are almost constant and not related to age. This is in accordance with another study that found similar SA concentration from birth to adulthood \(^{(10)}\). Meanwhile, this result is a disagreement with two other studies \(^{(11, 12)}\) that stated that SA in human unstimulated saliva was affected by age with a trend toward reduction in SA concentration with age. The result of this study is also a disagreement with Narhi et al. \(^{(13)}\) who stated that the concentration of SA and salivary peroxidase was highest in the oldest age group.

As mentioned, the results of the present study revealed low concentration of SA in the pre-ovulatory phase saliva; this is in accordance with two old studies that had observed decreased concentrations of SA both in human cervical mucus \(^{(14)}\), and human whole saliva in this phase \(^{(15)}\). Nadir concentration in the ovulatory phase was found in this study and this is in accordance with Moghissi and Syner \(^{(16)}\). Then a sudden rise to peak in the post-ovulatory phase; this is probably due to consumption of SA from the blood by the cell membranes of the growing follicles where it predominates because SA is a monosaccharide component of cell membranes \(^{(17)}\), so the consumed SA in the pre-ovulatory and ovulatory phases is more than the produced or gained. After the rupture of the dominant follicle “ovulation” takes place, the SA will be redelivered to the blood and the extra consumption of SA will be stopped in this phase. The fluctuation in SA concentration in the blood will be reflected in saliva. The results of the present study disagreed with Calamera et al. \(^{(18)}\)
who reported a peak in salivary SA concentrations in the pre-ovulatory phase and Alagendran et al. study (1) who reported a peak in salivary SA concentrations in the ovulatory phase.

**Glycosaminoglycans**

In the present study, GAG showed a comparable concentration in the ovulatory phase (0.412±0.175) ng/ml, to that of the control groups (0.452±0.279) for the pre-pubertal group and (0.445±0.041) for the postmenopausal group) with the peak GAG concentration in the post ovulatory phase. These results are so far away from Alagendran et al. (1) study, in which the GAG content showed a distinct peak at ovulation.

The present results are also a disagreement with Giampiero et al. (19), who stated that in the women with ovulatory cycles, plasma GAG levels differed significantly during menstrual cycle, with increased values at the periovulatory phase (3.5 µg/ml) with respect to the menstrual phase.

Another study did not find consistent variations during the normal menstrual cycle (20). In contrast, a significant variation in the urinary GAG concentration during ovulation was reported by Carranco et al. (21), this peak of maximal GAGs concentration (106.7 ± 46.2 micrograms/mL in urine) was noticed during the ovulatory phase.

In this research, the peak GAG concentration is in the post ovulatory phase (0.558± 0.218)ng/ml, this could be due to the release of follicular fluid with its GAG content after rupture of the follicle to the bloodstream which will be reflected in saliva.

Another possible cause to the reduced GAG concentration in the first half of the menstrual cycle, and its increase in the post-ovulatory phase is that heparenase enzyme (HSPE) is transiently induced by luteinizing hormone during the ovulatory process and may be down-regulated by the increasing progesterone levels in the luteal phase (22).

On the basis of the results arrived at, the study concluded that SA and GAGs were probably brought under the influence of cyclic variation of ovarian hormones. There are remarkable cyclic variations in sialic acid and GAGs during the menstrual cycle but in conclusion, GAGs and sialic acid cannot be used for the precise prediction of ovulation.

**REFERENCES**

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Figure 1: Saliva SA level in Control and Experimental Groups (mean± SD)

No significant correlation was found between SA and GAGs in all the study groups.

Figure 2: Saliva GAGs level in control and experimental groups (mean± SD)