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ANALYSIS OF LEVEL OF TOTAL CHOLESTEROL IN HUMAN FEMALE SALIVA UNDER DIFFERENT REPRODUCTIVE CONDITIONS AND METABOLIC DISORDERS.

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Abstract: Saliva is a complex diagnostic fluid, which contains a variety of enzymes, hormones, antibodies, antimicrobial constituents and growth factors. In our body fluids like blood, saliva, tears, sweat and urine are crucial resources of pathological diagnosis. Changing in level of total cholesterol level in human female saliva was investigated in 60 women volunteers during various stages of reproduction (pre-pubertal, parous, non-parous, menopausal and diabetogenic). According to findings, level of total cholesterol level is significantly increased during ovulatory phase, which was due to hormonal metabolic changes in the period of menstrual cycle.

Keywords: Saliva, Total cholesterol, prepubertal, parous, non-parous, menopausal and Diabetogenic.

Introduction:

Cholesterol is a waxy fat like substance found in cells of human body. It is an organic compound having 27 carbons with hydrocarbon tail, central sterol nucleus made up of four hydrocarbon rings and a hydroxyl group. It belongs to the steroid family and its molecular structure is $C_{27}H_{46}O$. It is an essential structural component of animal cells membrane and its major synthesis site is liver. It plays a role in forming and maintaining cell membranes and structures (**Krause, 2014**). It provides stability and fluidity. Cholesterol also plays a crucial role in regulating cell function (**Rahmati et al., 2019, Ding et al., 2019**). Total cholesterol includes low density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol. In human saliva variety of

electrolytes including calcium, magnesium, potassium, cholesterol, fatty acids, triglycerides, glycolipid and nitrogenous products are found (**Marini and Cabassi 2002, Actis et al., 2005, Agha-hosseini et al., 2006 and Caufal et al., 2003).** The concentrations of total lipids in parotid, submandibular and whole stimulated saliva were 0.2, 0.9 and 1.3 mg/dl respectively. Cholesteryl esters, cholesterol, triglycerides, diglycerides, monoglycerides and free fatty acids accounted for 96-99 percent of the total salivary lipids. Total Cholesterol is a direct precursor of steroid hormones, including corticosteroids, androgens, estrogens, progesterone and vitamin D, some of which are produced in the placenta (**Byanes, 2009)**. Cholesterol is essential for making a number of critical hormones, including the stress hormone cortisol. According to **Harvard publishing** Cholesterol is also used to make the sex hormones testosterone, progesterone, and estrogen. In women total cholesterol levels rise as estrogen levels increase during the monthly menstrual cycle and drop shortly before ovulation, then decrease more rapidly after ovulation occurs. High Total cholesterol level is one of the primary risk factors for heart disease, the leading cause of death among women (**Lloyd, 2009)**. The researchers found that as the level of estrogen rises, high-density lipoprotein (HDL) cholesterol also rises, peaking at the time of ovulation. HDL cholesterol is believed to be protective against heart disease. And when total cholesterol and low-density lipoprotein (LDL) cholesterol levels as well as another form of blood fat known as triglycerides declined as estrogen levels decline. Total cholesterol, LDL cholesterol and triglyceride levels reached their lowest just before menstruation began. **Alagendran et al., 2009** in their study assessed the usefulness of saliva as a biomarker of ovulation detection which showed that saliva can be used to test cholesterol and phospholipids instead of blood. **Al Rawi, 2010 & 2011** did two different studies and compared plasma and salivary lipid profile in individuals with ischemic heart stroke and the diabetes mellitus and suggested that lipid fractions particularly TGL can be assessed in saliva and may be used alone or in combination with other lipid parameters for monitoring disease activity and severity in such studies. The results of our study suggest that saliva can be used to assess, not only TGL but also TC, HDLC, VLDLC and to some extent LDLC. **Alagendran et al., 2009** conducted a study with 50 women between the ages 19 and 40 years and concluded that the lipid and its metabolites undergo consistent variations during the menstrual cycle. With significant review of literature 14 elevations of total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), phospholipids and triglycerides corresponding with peak estradiol levels at ovulation. High total and LDL cholesterol concentration was seen during the ovulatory phase and preovulatory phase of the menstrual cycle. The increased salivary HDL cholesterol levels in preovulatory phase may be attributed to decreased salivary triglycerides and reduced hepatic lipase activity. In a healthy person level of total cholesterol is less than 170 mg/dL. As women and men get older, their cholesterol levels rise. Before the age of menopause, women have lower total cholesterol levels. With high cholesterol, you can develop fatty deposits in your blood vessels. Sometimes, those deposits can break suddenly and form a clot that causes a heart attack or stroke. As earlier report of **Schwartz, 1981 and Mishell and Davajan 1979** indicated that during menopause ovulation fails to occur & female sex hormones diminish rapidly to almost none at all. In this condition non-utilization of cholesterol in steroidogenesis may be possible which increase the total cholesterol level in menopausal saliva in

women. As earlier report of **Meuram et al., 1998** and **Dodds and Dodds 1997** suggested that diabetes caused neuropathic changes in the salivary parenchyma with lymphocytic gland infiltrate. **Ship et al., 2002** also reported reduced salivary flow rate in uncontrolled diabetic patients. It was shown in a recent survey that only one in four women associates menopause with high cholesterol leading to a lack of awareness of the need to consider having cholesterol level checked around the time of the menopause (**Jackson, 2008).** A person's cholesterol levels can increase during or after menopause due to reduced levels of the hormone estrogen in the body. Salivary cholesterol and triglycerides of diabetic patients were estimated lower than that of non-diabetic patients. In contrast, **Priya et al. 2019** found a higher level of salivary lipids (cholesterol and triglyceride) in patients with type-1 diabetic. With the exception of Mycoplasma, which needs cholesterol for growth, it is not present in prokaryotes (bacteria and archaea).

Materials and Methods

The studies were performed in 60 different human female volunteers of age group (19 to 40 yrs.) categorized as prepubertal, parous, non-parous, menopausal and diabetogenic. Prepare each patient prior to collecting salivary sample. Patients must avoid chewing gum for at least 30 minutes prior to sample collection. In some cases, patients may need to fast overnight for 12-14 hours prior to sample collection.

1. Each patient brushed their teeth thoroughly without toothpaste.
2. Next, each patient had floss.
3. Rinsed patient's mouth with distilled water and sample collected.
4. Stored sample on ice.

Sample Preparation: Samples collected from each patient were centrifuged at high speeds and collected the supernatant. For immediate testing, stored supernatant on ice, otherwise stored supernatant at -20°C. Diluted the supernatant 25 to 100-fold with assay buffer (component B, Cat No. 40006). Added 50 µL/well of sample to test.

Results and Discussion:

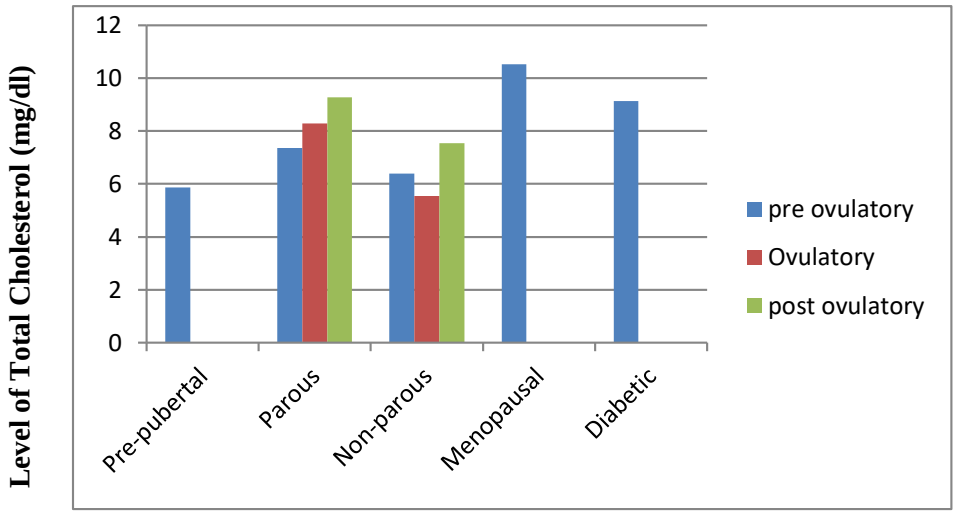
As the findings a highly significant ($p<0.001$) increased total cholesterol in parous ovulatory, post ovulatory, menopausal and diabetogenic condition in women's saliva was observed whereas highly significant ($p<0.01$) increased in parous preovulatory and significant ($p<0.01$) increased in parous preovulatory and significant ($p<0.02$) increased level in non-parous postovulatory were observed in comparison to prepubertal. It might be due to the active steroid synthesis by ovarian tissue than prepubertal stage. A highly significant ($p<0.001$) decreased total cholesterol level in non-parous preovulatory & ovulatory than parous preovulatory, ovulatory and post ovulatory was found whereas, highly significant ($p<0.01$) decreased level were observed in non-parous post ovulatory than parous post ovlatory. In menopausal women's saliva showed a highly significant ($p<0.001$)

increased level than parous preovulatory, ovulatory, postovulatory and non-parous preovulatory, ovulatory & post ovulatory phase.

Table 1: Level of Total Cholesterol in saliva of different conditions in human female subjects.

SL No.	Name of different conditions with symbols	Level of total cholesterol (mg/dl) mean ±SE of 5 samples	P-Value
1	**Pre pubertal –(a)**	**5.86 ± 0.265**	
2	**Parous** **I.Pre ovulatory –(b)** **I.Ovulatory – (c)** **I.Post ovulatory–(d)**	 **7.35 ± 0.105** **8.28 ± 0.076** **9.27 ± 0.258**	 a to b – (p<0.01) HS a to c – (p<0.001) HS a to d – (p<0.001) HS
3	**Non- parous** **I.Pre ovulatory-(e)** **I.Ovulatory-(f)** **I.Post ovulatory-(g)**	 **6.39 ± 0.167** **5.55 ± 0.153** **7.54 ± 0.425**	 a to g – (p<0.02) S
4	**Menopausal –(h)**	**10.53 ± 0.154**	a to h – (p<0.001) HS b to h –(p<0.001) HS c to h –(p<0.001) HS d to h – (p<0.01) HS e to h – (p<0.001) HS f to h – (p<0.001) HS g to h – (P<0.001) HS
5	**Diabetogenic –(i)**	**9.14 ± 0.316**	b to i–(p<0.001) HS c to i –(p<0.05) HS e to i – (p<0.001) HS f to i – (p<0.001) HS g to i – (p<0.02) S h to i – (p<0.01) S

Salivary total cholesterol might be possible to reproductive physiological disturbances and steroid hormones synthesis impairment whereas highly significant (p<0.01) decreased level is found than diabetogenic. Diabetogenic women's saliva showed a highly significant (p<0.001) increased total cholesterol than parous preovulatory, non-parous preovulatory and ovulatory phase and a highly significant (p<0.01) decreased level of salivary total cholesterol were observed than menopausal women saliva. A highly significant increased total cholesterol level in parous, non-parous, menopausal and diabetogenic women in comparison to prepubertal saliva might be an indication of its dependence on ovarian steroid hormone synthesis & secretion during ovulation.



Name of Different condition

Conclusion:

Lipids are one of the major constituents of the cell. Our body needs some cholesterol to work properly. But if We have too much cholesterol in our blood, we have a higher risk of coronary artery disease. An excessive cholesterol level can lead to cardiovascular diseases such as stroke, hypertension, elevated levels can cause serious problems. With high cholesterol, we can develop fatty deposits in our blood vessels. Eventually, these deposits grow, making it difficult for enough blood to flow through the arteries. Sometimes, those deposits can break suddenly and form a clot that causes a heart attack or stroke. Thus, saliva offers an alternative to serum as a biologic fluid for diagnostic purposes.

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