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RESEARCH ARTICLE

COMPARISION OF SERUM AND SALIVA COTININE LEVELS AMONG SMOKERS AND NON SMOKERS BY USING ELISA.

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Abstract

Background: Cotinine, important metabolite of nicotine, is a biomarker for monitoring tobacco exposure from both active smoking and passive smoking. The current study aimed to estimate the cotinine levels and compare the association between serum cotinine and salivary cotinine among smokers and non smokers. **Methods**: Consecutive consenting individuals (n=50) of 25 smokers (group I) & 25 non smokers (group II) with an age range of 21-75 yrs and with no apparent systemic illness were included in the study and detailed history of smoking was recorded. Serum and unstimulated saliva were collected from each subject in a separate plastic vials and cotinine levels were analyzed using Enzyme-linked immunosorbent assay (ELISA). **Results**: Among smokers 96% positivity for cotinine was shown in serum & 100% in saliva samples. Among non smokers 40% positivity for cotinine was shown in serum & 80% in saliva samples. When both groups were compared, cotinine positivity was slightly higher in saliva than serum. The results showed cotinine detection was more sensitive in saliva than serum samples. **Conclusion:** Saliva, being more accessible and noninvasive than serum, it can be used as an alternative diagnostic aid for detection of cotinine mainly for epidemiological surveys and patient education.

Introduction:-

Smoking has been linked with many deleterious effects on human health (Florescu A *et al*., 2009). The lack in the development of valid and precise method for measurement of exposure to smoking and its associated health risks constitute a dynamic area of research in the present era. Biomarkers are the most frequently used for ascertaining tobacco exposure. Nicotine was formerly considered as a definite biomarker as it is an important biologically active ingredient of tobacco. Nicotine biomarker assay is more sensitive due to its shorter plasma half life and presence of relatively low concentrations in the blood and urine among active and passive smokers (Kyerematen*et al*., 1990).

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Cotinine is an alkaloid and the major metabolite of nicotine; it can be used as an index for exposure to tobacco smoke and Environmental tobacco smoke (ETS) as majority (72%) of nicotine is transformed to cotinine (Benowitz NL, 1996; Benowitz NL *et al*., 2009). The cotinine plasma half-life is estimated to be nearly 15–20 hours where as nicotine half-life is no more than 0.5–3 hours (Jarvis MJ *et al.,* 1988). Due to its significantly longer half-life, cotinine biomarker is more often preferred than nicotine for the measurement in blood serum, urine, semen, hair and saliva. The half-life of cotinine in serum and saliva is approximately the same (Benowitz NL, 2009; Kunzle R *et al*., 2003; AL-Delaimy WK, 2002).

To study a disease process associated with smoking, saliva is considered as important diagnostic tool. In addition, to the investigation of saliva for exposure of tobacco smoke, it also provides vital information regarding the functioning of a variety of organs and endocrine system inside the body. With respect to epidemiological approach saliva is quite stable at surrounding temperature which in assays saliva provides a newer instrumentation (Chiappin S *et al*., 2007). Studies also suggested that saliva cotinine estimation can be future alternative to serum, although serum is more sensitive to early changes in smoking status than saliva cotinine (Haley NJ *et al*., 1983; Sepkovic DW *et al*., 1985). Other methods proposed for the assessment of cotinine levels in biological matrices includes gas (GC) and liquid (LC) chromatography to observe the exposure in tobacco smokers (Jacob P *et al*., 1992; Malafatti L *et al*., 2010). Due to expensive cost of equipment, sampling methods and expertise in the field, GC and LC are not always suitable for the evaluation of cotinine levels (Jacob P *et al*., 1992; Matsumoto A *et al*., 2010). ELISA is an alternative procedure for a larger sample size where samples are analyzed rapidly or when suitable chromatographic equipment is unavailable. Reports suggest that immunoassays are more advantageous for comparative values than analytical equipment systems (Szumska M *et al*., 2013; Matsumoto A *et al.,* 2010; Benkirane S *et al*., 1991).

Cotinine has a smaller molecular size, negligible protein binding in blood and relatively water soluble with a concentration of 15% to 40% more in saliva than serum (Avila-Tang E*et al.,* 2012). Thus cotinine measurement in saliva becomes an easy, non invasive method of estimation for larger samples in a limited time period (Avila-Tang E*et al.,* 2012; Wall AM *et al*., 1998; Nosratzehi T *et al*., 2015).

To maintain uniformity in the method of sample collection and storage, some researchers have an inclination to use unstimulated, whole saliva that pools on the floor of the mouth (Granger AD *et al*., 2007). Considering this background, the aim of this study is to estimate the cotinine levels and compare the association between serum cotinine and salivary cotinine among smokers and non smokers.

Materials and Methods:-

A total of 50 individuals in our locality (Tirupathi) were enrolled. The subjects were equally divided into two groups consisting of 25 smokers (group I) & 25 non smokers (group II) with an age range of 21-75 yrs and with no apparent systemic illness were included in the study and detailed history of smoking was recorded. Written informed consent was obtained from all the subjects who were enrolled for the study. Among smokers 25 males who smoked two to fifty cigarettes per day were enrolled & among 25nonsmokers; 21 were males and 4 females, who did not smoke but either lived with a smoker or worked in an office or ward where smoking was allowed. Ethical clearance was obtained &both serum and unstimulated saliva samples were collected from each individual and subjected to cotinine analysis by ELISA. The serum samples from venipuncture and unstimulated whole saliva samples were collected from each group in two separate vials, labeled &immediately frozen at -20^0C until further evaluation of cotinine by ELISA was done by using the respective kits (serum cotinine- KRISH LIFE:9001 and saliva cotinine-SALIMETRICS:2112). Saliva cotinine values >0.1ng/mL and serum cotinine values >1ng/mL were labeled as positive for cotinine.

Results:-

The study included 50 subjects which were divided into two groups. The mean age of nonsmokers was 47.24±10.50 (27-71) years, and the smoker's was 52.68±12.52 (26-73) years (Fig 1). Among smokers, 24 (96%) samples were positive for cotinine in serum and all the 25 (100%) samples were positive for saliva (Fig 2). Among non-smokers 10 samples were positive for serum whereas 20 samples were positive for saliva (Fig 3). Comparison of serum and salivary cotinine was done using Two-way ANOVA test. When both the groups were compared smokers showed increase in serum and salivary cotinine levels than non smokers and was statistically significant ($p<0.001$) (Fig 4).

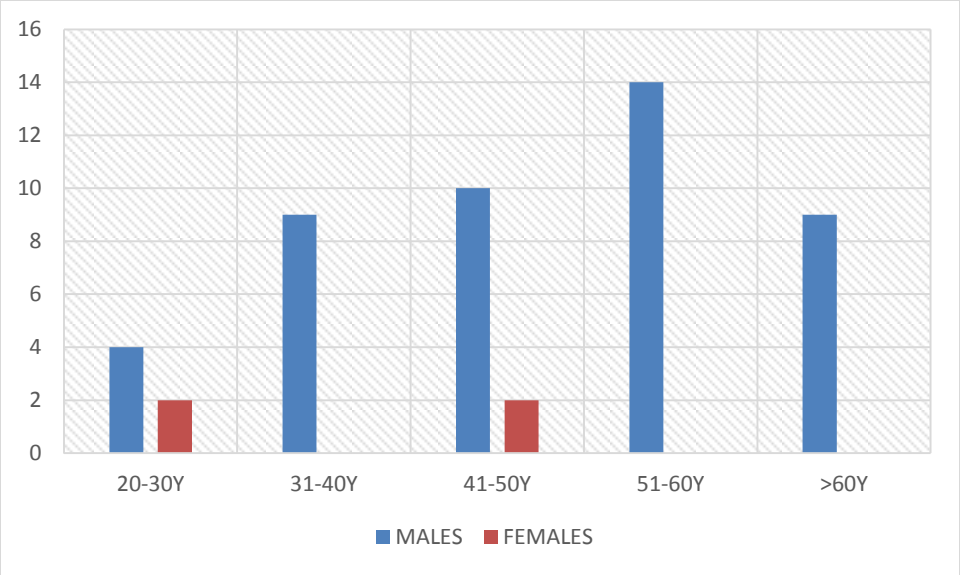

Fig 1:-Age wise distribution among smokers and non smokers

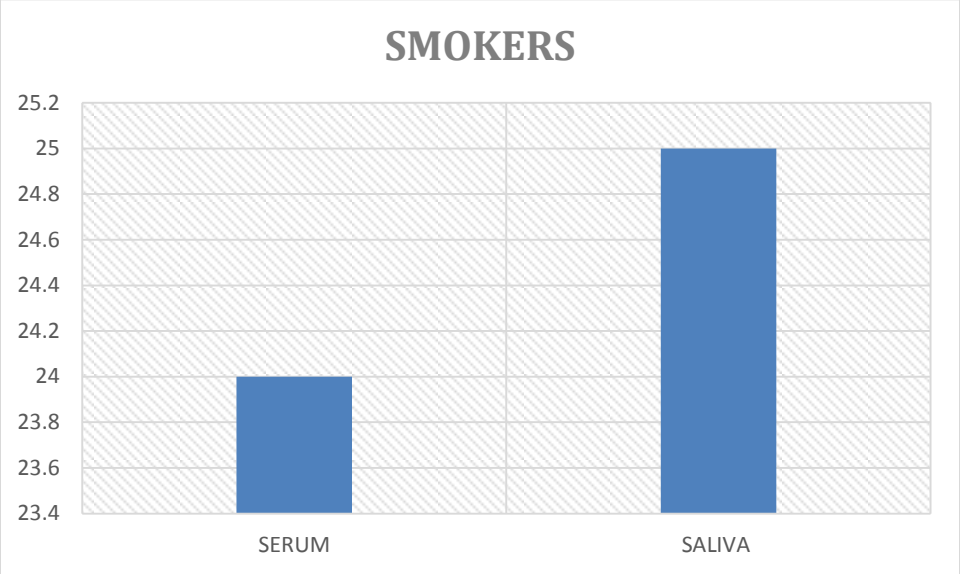

Fig 2:-Comparison of Serum and Saliva cotinine positive subjects in Smokers

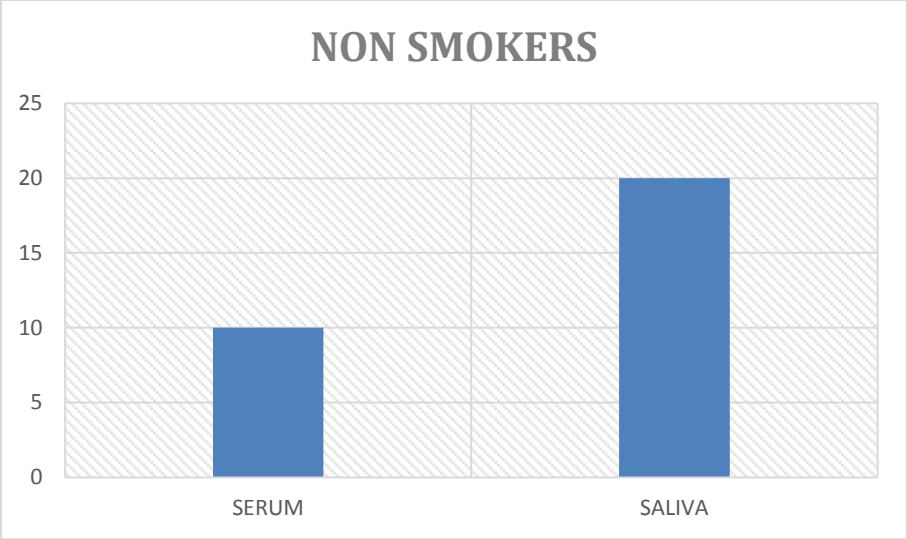

Fig 3:-Comparison of Serum and Saliva cotinine positive subjects in Nonsmokers

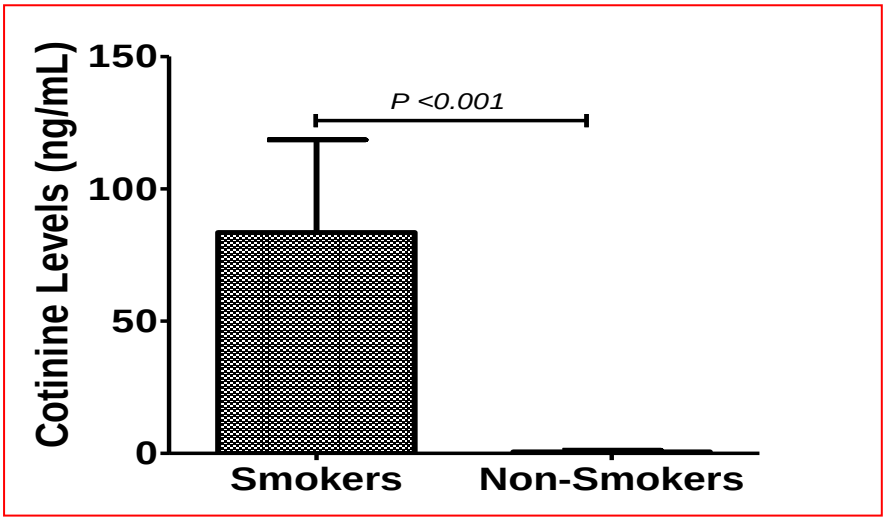

Fig 4:-Overall comparison of cotinine levels in Smokers and Non-Smokers

Discussion:-

Tobacco smoking accounts for 70% of chronic lung diseases (Johnson NW *et al*., 2000). Furthermore, in passive smokers, tobacco smoke which they inhale accounts for formation of free radicals and many toxic substances and the concentration of these harmful substances in passive smoking is relatively greater than in the smoke inhaled by a smoker which many people are not aware of. (Dvorak RD *et al*., 2008).

During epidemiological surveys and patient education, exposure of tobacco smoke was mostly evaluated via a self-administered questionnaire (Malgorzata HJ *et al*., 2012; Etter JF *et al*., 2001). Since, self-administered questionnaire is not a good assessment for the exposure of smoke, quantitative assessment of metabolites of tobacco smoke exposure would help. Cotinine with its high specificity and retention period in the body fluids in sufficient quantifiable levels it is more preferred for quantifying tobacco smoke exposure (Watts RR *et al*., 1990). Cotinine, because of its longer half-life and higher specificity in separating smokers from nonsmokers as well as in evaluating day to day smoking behavior is considered as the best biomarker (Chadwick CA *et al*., 2007). Cotinine is found in higher quantity in non-ionized form in the blood (pH 7.4) due to its pKa (4.5), and poor solubility of the free base form in lipids resulting in lower distribution to tissues, which can explain their extended half-life in blood. Another factor that prolongs cotinine half-life is relative low rate of renal excretion than nicotine (Feyerabend C *et al* 1980). Cotinine concentrations in saliva remains stationary even after several hours due to selective accumulation of

cotinine by the salivary glands (Sepkovic DW *et al*., 1985). A study done by Wall AM *et al*., 1988 on cotinine in the serum, saliva and urine among active smokers, passive smokers and nonsmokers showed that cotinine can be estimated from all body fluids could distinguish active smokers from other groups.

Tobacco smoke exposure is classically recognized as a most important environmental hazard for periodontal diseases (Arbes SJ *et al*., 2001). The specific association between smoking and periodontitis is evident from the environmental studies (Brut B, 2005; Johnson N *et al*., 2000).

A study conducted by Bernert et al**.,** 2000, showed that the unstimulated salivary cotinine levels are more closely correlated to serum cotinine levels than with stimulated saliva. Whole unstimulated saliva more advantageous as it enables collection of large sample volume and minimizes the influence of substances used to collect. It can also be assayed for multiple markers and allows unused sample to be frozen in an archive for future assay (Granger DA *et al*., 2007). Hence, unstimulated salivary cotinine levels were assessed and compared to serum cotinine levels in the current study.

In our study, smokers have shown cotinine positive levels in both serum (96%) and saliva (100%) and in nonsmokers, serum samples (40%) and 20 saliva samples (80%). The values of cotinine levels showed a significant difference ($p < 0.001$) among smokers and nonsmokers. Salivary cotinine levels were shown more positive than serum in both groups. This indicates that minor exposure to tobacco smoke can be detected in saliva samples.

In our study, among nonsmokers, 2 female subjects and 18 male subjects showed cotinine positivity in saliva samples, who have been exposed to passive tobacco smoke due to smoking habit of family members/work place. ETS cause pregnant women have an increased risk of having low-birth weight babies, increased risk of breast cancer & pneumonia etc. The passive exposure to tobacco smoke can significantly increase oxidative stress parameters and young people who are exposed to environmental tobacco smoke are often not aware of the fact that frequent passive exposure may lead to significant increase in cancer risk. An investigation done by Etter JF *et al*.,2000 on salivary cotinine levels showed 1.5 times higher concentration in nonsmokers whose close friends/spouses were smokers.

Conclusion:-

In the present study we identified increased cotinine positivity for saliva than serum. Among passive smokers, salivary cotinine showed higher positivity than serum cotinine. Further cross sectional studies has to be done by using larger sample size. Though, chromatographic methods are more specific for evaluation, due to its expenses and availability, salivary cotinine estimation by ELISA can be used in epidemiological surveys as patient education and assessment tool for tobacco smoke exposure.

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