Original Research Paper

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# Comparison of urinary cotinine and psychiatric morbidity

Dental Science

**KEYWORDS:** Cotinine , Tobacco industry, Addiction, Smoking Caused Disease

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## ABSTRACT

**Background and Objective:** Tobacco and its various forms are widely used in modern era within diverse age groups by both genders. The harmful effects of tobacco as an etiopathogenic agent are widely acknowledged. Research work on nicotine exposure and psychiatric morbidity is limited. Urinary cotinine level gives precise evidence of nicotine dependence and general health questionnaire -28 enables to gage mental health of a subject. The aim of the study is to compare psychiatric morbidity and urinary cotinine levels of tobacco users. **Methods:** A total number of 25 smokers, 25 tobacco chewers, full filling inclusion and exclusion criteria, were assessed with a control group of 25 subjects not having any habits, were evaluated for psychiatric morbidity and urinary cotinine level measurement. General health questionnaire-28 was applied for all subjects to measure psychiatric morbidity. Reverse phase high pressure liquid chromatography was used for estimation of urinary cotinine level. **Results:** Psychiatric morbidity (mean) was three times higher in smokers and tobacco chewers when it was compared with control group. Urinary cotinine level (mean) was 401 times higher in tobacco chewers, and 244 times higher in smokers when compared to control groups. Both psychiatric morbidity and urinary cotinine levels were significantly higher in tobacco users compared to control group. **Conclusion:** Psychiatric morbidity and urinary cotinine levels are significantly higher in tobacco users in comparison with non-users of tobacco.

**INTRODUCTION:**

Tobacco cultivation has a history of about 8000 years. Tobacco is consumed both in smoking and non-smoking forms. Smoking forms include cigarette, bidi, hooka and chutta (a reverse form of smoking in which smoking is done with the burning end inside the mouth).[1] Tobacco use is the single largest preventable cause of mortality and morbidity globally.[2] Tobacco kills a third to half of those who use it, as tobacco use has been associated with various carcinomas including lung, esophagus, larynx, mouth, throat, kidney, bladder, pancreas, stomach, and cervix. Around the world, smoking is responsible for 90% of all cases of lung cancer.[3] Tobacco control continues to be one of the major health challenges of modern times. The prevention of tobacco use in young people is the single greatest opportunity for preventing non-communicable disease in the world today.[4]

Precise estimation of exposure to tobacco smoke is a concern for clinicians as well as for epidemiologists. Concerns have been expressed about the reliability of self-report on use of tobacco products.[5]

Many biomarkers are available for the determination of tobacco exposure, nicotine and cotinine stand out to be the most easily identifiable markers. Although nicotine is a desirable biomarker for tobacco consumption status, there are limitations to its utility: a short half-life, complex measurement, and variability in plasma levels (which might be higher in subjects with impaired kidney excretion function). Cotinine, a metabolite of nicotine, can be measured in saliva, plasma, and urine. Cotinine has relative advantages over other biomarkers—a longer half-life (about 18-20 hours), convenience of measurement, and little daily variability in chronic smokers, stable in body fluids, has a long half-life, low plasma protein binding (2.6%), and dose independent disposition kinetics. These factors make cotinine a good marker for estimating both active and passive exposure, therefore, its level has been regarded as a useful objective measure for smoking status or exposure. Urinary cotinine level is a widely used biomarker. A good correlation exists between urinary cotinine and daily tobacco consumption.[6]

The pharmacologic effect of nicotine plays a crucial role in tobacco addiction.[7] When issues about tobacco use are considered, "nicotine addiction" can be regarded as a roadblock that needs to be overcome. The importance of nicotine in maintaining smoking and in cessation difficulty has been well acknowledged.

General Health Questionnaire (GHQ) is a screening device for identifying minor psychiatric disorders in the general population and within community or non-psychiatric clinical settings such as primary care or general medical out-patients. Suitable for all ages from adolescent onwards – not children, it assesses the respondent's current state and asks if that differs from his or her usual state.[8]

By precisely estimating urinary cotinine level for tobacco users one can correctly predict the severity of the addiction and by taking the score from the general health questionnaire, current mental status of the tobacco consumer can be specifically estimated. In present study, we aimed to evaluate tobacco usage through cotinine measurement and psychiatric morbidity through general health questionnaire.

**AIMS OF THE STUDY:**

Aim of the study was to compare psychiatric morbidity and urinary cotinine levels of the tobacco users.

**STUDY DESIGN:**

Study was conducted on subjects visiting the S.D.M College of Dental Sciences and Hospital, Dharwad.

A) Subject inclusion criteria:

1. Patients exposed to smokeless and smoking tobacco as a daily habit (at least one smoke/tobacco chewing per day for one year).

B) Subject exclusion criteria:

1. Patients exposed to any other types of smoking (cannabis, opium).

2. Undergoing antibiotic or steroid treatment.

3. Pregnant and lactating mothers.

4. Systemic disorders associated with psychological morbidity

Participants were informed about the procedure and written informed consent was obtained. A total of 50 subjects of both the genders, above the age of 18 years and fulfilling the above inclusion and exclusion criteria were selected for the study. All subjects were asked about their habit history and were divided into two groups based on tobacco consumption (smoking/smokeless). Patients were interviewed with a semi structured pro-forma to know sociodemographic and clinical details. The details about the frequency duration about their habit history was taken. Control group consisted of 25 subjects of age and gender matched

individuals, who did not consume tobacco in any form but have dental disease like periapical pathology, pericoronitis, gingivitis, partial edentulism and malocclusion.

Psychiatric morbidity:
General Health Questionnaire-28 was administered to evaluate psychiatric morbidity. For people who did not know English, translated version of GHQ 28 in Kannada language was taken.

Urinary cotinine level estimation:
Urine samples were collected and urinary cotinine levels were assessed by high pressure liquid chromatography.

Sample Preparation: Prior to analysis, the urine samples (0.5ml) was added with internal standard, alkalinized by NaOH and extracted with dichloromethane. The organic fraction was evaporated using a rotary evaporator at 40 ºC. The residue was re-dissolved in mobile phase consisting of KH2PO4 and sodium 1-heptane sulfonate. An aliquot of this mixture was used for injection into the HPLC system.

Chromatography: The quantification of Cotinine was done using Reverse Phase-HPLC technique. Throughout the study, all reagents and standards used were of analytical grade and solvents were of chromatography purity. Liquid chromatography was carried out on an Agilent 1100 (Agilent Technologies) series high performance liquid chromatography system equipped with vacuum degasser, pump, manual injector and a variable wavelength UV-Visible detector (Agilent Technologies). Separation was carried out on an Agilent Zorbax C-18 analytical column, which served as stationary phase (100mm X 4.6mm, 5um particle size). Mobile phase consisting of sodium 1-heptane sulfonate, $KH_2PO_4$, methanol and water was used for isocratic elution. Further chromatographic conditions such as mobile phase flow rate, pH, sample injection volume, column temperature was optimized. Under optimized conditions, identification of peaks were based on comparison of chromatographs of standards with that of calibrators and biological samples.

Data acquisition and interpretation: was performed on ChemStation LC software integrated with the chromatography system.

**STASTICSAL ANALYSIS:**
For comparison among groups 'Chi square test' and 'One way ANOVA' was applied. Pair wise comparison was done by 'Turkeys multiple posthoc procedure'. Non parametric tests were applied to check whether 3 groups differ significantly using 'Kruskal Wallis' and 'Mann Whiteney U test'. Pearson's correlation was taken to evaluate significance of the parameters within subjects of each group.

**RESULTS:**
First we analyzed the age of the patient and then calculated the mean age of the patients, mean age for the control group was 32.44, for smokers it was 29.56 and for tobacco chewers it was 29.24 years.

The chronicity of the habits of the subjects were measured on the grade of frequency. It was assessed by asking about their habit history and then it was divided into 4 groups, none- as control group, subjects away from the tobacco use, upto 5 times a day, between 5 to 10 times a day, and upto 10 - 15 times or more than 15 times a day. The analysis showed wider distribution frequency between 5 – 10 times a day in both smokers and chewers group.

| Groups | Mean | SD | SE |
|---|---|---|---|
| Control group | 32.44 | 9.88 | 1.98 |
| Smokers group | 29.56 | 8.13 | 1.63 |
| Tobacco group | 29.24 | 8.39 | 1.68 |
| Total | 30.41 | 8.83 | 1.02 |

Table 1: Summary statistics of age in three groups (control, smokers and tobacco chewers)
GHQ score:

When the score showing psychiatric morbidity was analyzed the mean score of smokers and tobacco chewers had significant difference with control group. The mean GHQ score was highest in smokers followed by tobacco chewers, GHQ score of controls was considerably less.

**Table 2:** Summary statistics of GHQ scores in three groups (control, smokers and tobacco chewers)

| Groups | Mean | SD | SE |
|---|---|---|---|
| Control group | 5.52 | 3.71 | 0.74 |
| Smokers group | 15.20 | 5.71 | 1.14 |
| Tobacco group | 13.96 | 5.71 | 1.14 |
| Total | 11.56 | 6.66 | 0.77 |

**Table 3:** Pair wise comparison of three groups (control, smokers and tobacco chewers) with GHQ scores by Tukey's multiple posthoc procedures

| Groups | Control group | Smokers group | Tobacco group |
|---|---|---|---|
| Mean | 5.52 | 15.20 | 13.96 |
| SD | 3.71 | 5.71 | 5.71 |
| Control group | - | | |
| Smokers group | p=0.0001* | - | |
| Tobacco group | p=0.0001* | p=0.6706 | - |

**URINARY COTININE LEVEL:**
Urinary cotinine level obtained from the HPLC showed a significant difference among the smokers and control group and also among the tobacco chewers and control groups. It was highest in tobacco chewers followed by smokers. Control group had a very low mean score of urinary cotinine level.

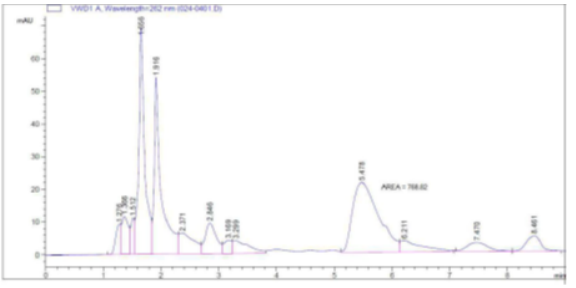
**Table 4:** Summary statistics of concentration (ng/ml) scores in three groups (control, smokers and tobacco chewers)

| Groups | Mean | SD | SE |
|---|---|---|---|
| Control group | 5.85 | 3.53 | 0.71 |
| Smokers group | 1346.44 | 727.24 | 145.45 |
| Tobacco group | 2217.64 | 851.37 | 170.27 |
| Total | 1189.98 | 1115.96 | 128.86 |

**Table 5:** Pair wise comparison of three groups (control, smokers and tobacco chewers) with concentration (ng/ml) scores by Turkey's multiple posthoc procedures

| Groups | Control group | Smokers group | Tobacco group |
|---|---|---|---|
| Mean | 5.85 | 1346.44 | 2217.64 |
| SD | 3.53 | 727.24 | 851.37 |
| Control group | - | | |
| Smokers group | p=0.0001* | - | |
| Tobacco group | p=0.0001* | p=0.0001* | - |

Tobacco chewers: Sample: 1 Urinary cotinine concentration = 833.02 ng/ml



**Smokers: Sample: 1:** Urinary cotinine concentration = 1471.30

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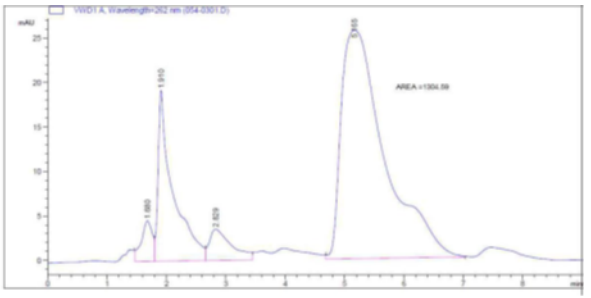
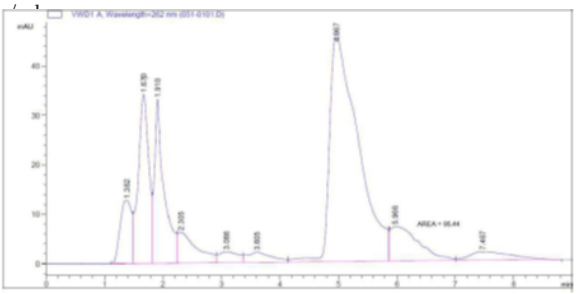
ng/ml



**Control group: Sample: 1:** Urinary cotinine concentration: 6.60



**DISCUSSION:**

Tobacco use increases with increasing age. It is seen that in areas with a high prevalence of tobacco use, initiation may occur at an early age. The National Household Survey of Drug and Alcohol Abuse in India (NHSDAA), conducted in 2002 among males, covering over 40,000 individuals aged 12-60 years in nearly 20,000 households in 25 states revealed that the overall prevalence of current tobacco use was 55.8% showing an increase in tobacco use with age, levelling off after 50 years of age.[9] Tobacco may be used in raw, processed mixtures and pyrolised forms. The raw forms are generally sun-cured or air-cured, consist of flakes of plain tobacco leaves mixed with other ingredients especially lime, areca nut and / or other condiments. The pyrolised forms (mishri, bajjar, etc.) are used as dentifrice. Oral use of snuff is also practiced in some specific areas.[10]

Consumption of tobacco in smoking form has a diverse pattern. It greatly depends on the social parameter of the subjects who consume it. Cigarette, bidi, hooka and cigar the most prevailing forms of smoking in India. A specialized form reversed smoking is also seen among the subjects.

WHO estimated that globally over 450 million people suffer from mental disorders. Currently mental and behavioural disorders account for about 12 percent of the global burden of diseases. This is likely to increase to 15 percent by 2020. In India the burden of mental and behavioural disorders ranges from 9.5 to 102 per 1000 population. Encumbrance of mental disorders seen by the world is only a tip of iceberg. Burden of mental disorders had risen over last few decades. Mental health is a state of well-being in which the individual realizes his or her own abilities, can cope with the normal stresses of life, can work productively and is able to make a contribution to his or her community. Major proportions of mental disorders come from low and middle income countries.[11]

Work environment, school environment and family environment plays important role in pathogenesis of mental disorders.[12] It is widely recognized that psychiatric morbidity covaries with psychoactive substance use among adolescents [13] as well as adults.[14]

For example, it has been estimated that individuals with psychiatric disorders purchase approximately 44 percent of all cigarettes sold in the United States, which undoubtedly contributes to the disproportionate rates of morbidity and mortality in these populations. In young smokers, the behavior appears to be strongly associated with increased risk for a variety of mental disorders. In some cases, such as with conduct disorders and attention-deficit hyperactivity disorder, these disorders may precede the onset of smoking, while in others, such as with substance abuse, the disorders may emerge later in life. Among adults, the rate of major depressive episodes is highest in nicotine–dependent individuals, lower in nondependent current smokers, and lowest in those who quit or never started smoking. Adult tobacco use also increases risk for the later development of anxiety disorders, which may be associated with an increased severity of withdrawal symptoms during smoking cessation therapy. Tobacco contains nicotine, a highly addictive chemical which is quickly absorbed into the bloodstream. Nicotine in-turn stimulates dopamine production, a chemical associated with pleasurable feelings.[15]

A study conducted to examine association of smoking and nicotine dependence with psychiatric morbidity, controlling the potential confounding effect of smoking on relationship between the use of the other substances and psychiatric morbidity. Information was collected using structured questionnaire that included information on the sociodemographic characteristics consumption of tobacco, alcohol, caffeine and illegal drugs, psychiatric morbidity. Spanish version of the General Health Questionnaire was used to detect psychiatric morbidity. Subjects were classified for psychiatric morbidity defined by General Health questionnaire. They showed strong association between tobacco exposure and GHQ score. Bivariate analysis with chi-square tests used to determine the associations of psychiatric morbidity with other variables which showed high nicotine dependence in a significant association with both the GHQ score and psychiatric morbidity and with self-report of any mental disorder (non-psychotic).[16]

Whenever tobacco is consumed in any form, nicotine-rich blood passes from the lungs to the brain within seven seconds and immediately stimulates nicotinic acetylcholine receptors; this indirectly promotes the release of many chemical messengers such as acetylcholine, norepinephrine, epinephrine, arginine vasopressin, serotonin, dopamine, and beta-endorphin in the brain. Nicotine also extends the duration of positive effects of dopamine and increases sensitivity in brain reward systems. Nicotine is extensively metabolized to a number of metabolites- cotinine, trans-3'-hydroxycotinine (3-OH-cotinine), and nornicotine. Nicotine has very low half-life and its detection in the blood becomes very difficult. Cotinine has an in vivo half-life of approximately 20 hours, and is typically detectable for several days (up to one week) after the use of tobacco. The level of cotinine in the blood, saliva, and urine is proportionate to the amount of exposure to tobacco smoke, so it is a valuable indicator of tobacco exposure, including secondary (passive) smoke. Cotinine is a major metabolite of nicotine, urinary excretion of cotinine is a good marker as it is less influenced by the flow of urine and pH. A study conducted to evaluate urinary levels of nicotine and cotinine in tobacco users. The study estimated urinary levels of nicotine and cotinine in healthy individuals using different types of tobacco to identify and validate the smoking status. 24 hour urine sample of 130 healthy volunteers (smokers=70, passive smokers=20, tobacco chewers=20, non-smokers=20) were analyzed by high pressure liquid chromatography assay. The mean value of nicotine (ng/ml) and cotinine (ng/ml) in urine were highest in cigarette smokers (nicotine=703.5+/-304.34; cotinine 2739.20+/-983.29), in passive smokers (nicotine=109.75+/-22.33, cotinine 280.75+/-86.30) and in non-smokers the values were much lower (nicotine 55.0+/-13.71; cotinine 7.30+/-2.47). All forms of tobacco users had a significantly higher values compared to passive smokers and non-users.[17]

The concentration difference depends on the frequency, concentration of nicotine in the tobacco product, physiological events, such as meals, posture, and exercise. During sleep hepatic blood flow declines and nicotine clearance falls correspondingly, nicotine and cotinine clearances are higher in women than in men, drugs such as rifampicin, phenobarbitone affect the metabolism of nicotine. Smoking itself increases the metabolism of nicotine,

clearance of cotinine was significantly lower in blacks than in whites, and renal clearance affects cotinine levels significantly. Renal clearance of cotinine is much less than the glomerular filtration rate. Since cotinine is not appreciably protein bound, this indicates extensive tubular reabsorption. Renal clearance of cotinine can be enhanced by up to 50% with extreme urinary acidification. Cotinine excretion is less influenced by urinary pH than nicotine because it is less basic and, therefore, is primarily in the unionized form within the physiological pH range. Renal excretion of cotinine is a minor route of elimination, averaging about 12% of total clearance.[18]

We found out the mean GHQ score of all the groups and the results showed Control group with score of 5.52, Smokers group with score 15.20, and for Tobacco chewers group the score was 13.96. GHQ score of tobacco chewers and smokers is found to be three times higher than the GHQ score of the control group. One way ANOVA and Turkey's multiple posthoc procedures showed a substantial difference between the groups, which was showing significant p-value. Pair wise comparisons between control & smokers group and control & tobacco chewers group revealed significant difference (p=0.0001), but the pair wise comparison among smokers and tobacco chewers was not statistical significant (p=0.6706). Urinary cotinine level, controls had levels of 5.85 ±3.53 ng/ml, for smokers it was 1346.44 ± 727.24 ng/ml and for tobacco chewers 2217.64 ± 851.37 ng/ml. These finding clearly suggests us that urinary cotinine level is 401 times higher in tobacco chewers and 244 times higher in smokers compared to control group. One way ANOVA and Turkey's multiple posthoc procedures showed a substantial difference between the groups which was showing significant p-value. Pair wise comparisons between control & smokers group and control & tobacco chewers group revealed significant difference(p=0.0001), unlike the GHQ score pair wise comparison, divergence for urinary cotinine level among smokers and tobacco chewers the difference was statistically significant(p=0.0001).

During conversations, tobacco chewers and smokers usually do not give clear picture about frequency and duration of tobacco consumption. They often misguide the clinician with the false number. Urinary cotinine level gives precise estimation the nicotine exposure of the patient for last 48 hours. Urinary cotinine level give us a result about cumulative, unbiased and transparent estimation of nicotine exposure, similar like HBA1C estimation for diabetics.

The present study has clearly established a correlation between psychiatric morbidity and urinary cotinine level among smokers and tobacco chewers when we compared them to control group. The overall psychiatric morbidity and urinary cotinine levels were significantly higher in tobacco chewers and smokers.

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