

# Correlation of Cotinine Levels with Use of Smokeless Tobacco (Mishri) among Pregnant Women and Anthropometry of Newborn

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## ABSTRACT

**Introduction:** 'Smokeless tobacco' is the term used for the tobacco that is consumed in un-burnt form and it can be used orally or nasally. Cotinine, a nicotine metabolite, is used to quantify exposure to tobacco, which readily gains access to foetal circulation. Cotinine is invariably found in coelomic, amniotic and foetal serum when maternal serum cotinine levels exceed 25ng/ml.

**Aim:** To estimate cotinine levels among pregnant women using and not using smokeless tobacco (mishri) and to correlate cotinine level with anthropometry of newborns.

**Materials and Methods:** A hospital based cohort study was conducted at Krishna Hospital, Karad, District Satara,

Maharashtra, India. Pregnant women who were using smokeless tobacco (mishri) during pregnancy were analyzed for cotinine levels in blood by using ELISA kit tech and correlated with anthropometry of newborn babies and compared with non users of tobacco.

**Results:** About 480 gm reduction in Birth weight and 6.5 cm reduction in birth length of babies born to mishri users compared to non users of tobacco and also cotinine levels among users were found significantly negatively correlating with anthropometric measurement of newborn babies.

**Conclusion:** A pro-active effort is essential to educate the women about adverse effects of tobacco in general and on the intrauterine growth of the baby in particular.

**Keywords:** Birth weight, Birth length, Intrauterine growth, Nicotine metabolite

## INTRODUCTION

'Smokeless tobacco' is the term used for the tobacco that is consumed in un-burnt form and it can be used orally or nasally [1-3]. One-third of population consume smokeless tobacco regionally, in traditional form like betel quid, tobacco with lime and tobacco tooth powder etc., [4]. In different parts of India, prevalence of use of tobacco as dentifrice varies from 6% to 68% [5,6].

Mishri (masheri or misheri) is one of the smokeless tobacco used as dentifrice that is prepared by roasting of tobacco on a hot metal plate until it is uniformly black and is then made in to powder. It is taken from packet or metal container with the index finger and applied to teeth and gums for the purpose of cleaning the teeth but later on addiction is caused due to addicting properties of tobacco. This habit is common in Maharashtra with prevalence of 39% among women and 0.8% among men [1], a recent study showed frequency of mishri use among women as 68.69% [7].

Cotinine, a nicotine metabolite, is used to quantify exposure to tobacco, which readily gains access to foetal circulation. Foetal cotinine concentration in pregnant smokers is on an average 90% of maternal values throughout gestation and these values remain higher in foetal circulation for longer period [8,9]. Cotinine is invariably found in coelomic, amniotic and foetal serum when maternal serum cotinine levels exceed 25 ng/ml [10].

A general impression prevails that ill effects of tobacco are related to smoking but the effects of smokeless form of tobacco consumption are underestimated. There are many studies on effects of smoking on pregnancy and its outcome whereas, studies on smokeless tobacco are relatively fewer in India. A study was undertaken on pregnant women to find out effects of mishri on the mothers and the babies born to them. The aspect of correlation of mishri use and the levels of haemoglobin are already published [11]. This article is being published on the effects of mishri use by the mothers on the anthropometry of their newborn babies. Considering the

high proportion of Low Birth Weight (LBW) babies among Indian population a study of this kind on correlation between smokeless tobacco and LBW becomes important to reduce the morbidity in mothers and their babies associated with tobacco consumption during pregnancy.

## MATERIALS AND METHODS

A hospital based cohort study was conducted at Krishna Hospital, Karad, District Satara, Maharashtra, India in 2011. Study included the pregnant women who delivered at Krishna Hospital, Karad during the study period. Pregnant women who were using smokeless tobacco (mishri) during pregnancy were included as study subjects and those pregnant women who were not using any form of tobacco were included as controls after matching them for age and parity [11].

Pregnant women who delivered twin babies and having any major illness like diabetes, malaria, tuberculosis, HIV/AIDS etc., affect the pregnancy and its outcome were excluded.

Sample size for this purpose was obtained by calculation based on previous study as follows; to obtain mean difference in birth weights of 170 gm [12] (2,750 gm±344.0 vs. 2,580 gm±275.6) among users and nonusers of mishri with permissible error 10% and 95% of confidence limits, power 95%, it came around 88+10% attrition i.e., 97 minimum in each group by using the formula  $n=(SD1^2+SD2^2) \times (Z_{1-\alpha/2} + Z_{1-\beta})^2 / d^2$ .

Detailed history was taken from the subjects and from the case records as per the pretested proforma on the day of delivery, which included personal details, diet history, obstetric history (present and past), history of parturition, anthropometric measurements of newborn baby among users and non users of mishri. The details of mishri use were obtained from the users. A sample of mishri indicative of the amount used each time was obtained from each subject and then weighed by using standard electronic weighing machine with 2 gm least count in Biochemistry laboratory.

Maternal blood (1 ml) of study subjects and controls was collected in a plain bulb within 24 hours of delivery and was processed in Biochemistry laboratory of Krishna Institute, which included centrifugation, collection of serum in pendrop (plastic bulb), estimation of cotinine in serum by using ELISA kit method. Then the levels of cotinine in serum were recorded in the respective proforma of respective participants (study subjects and controls) in nanogram per millimeter (ng/ml). Standardization of method of estimation of cotinine was done before study. Institutional Ethics Committee permission was obtained and an informed consent of the study participants was taken.



[Table/Fig-1]: ELISA kit for estimation of cotinine in blood.

The kit in the study used was a solid phase competitive ELISA. In the wells coated with anti-cotinine antibody, the samples and cotinine enzyme conjugate were added. Cotinine present in the samples has to compete with a cotinine enzyme Horse Radish Peroxide (HRP) conjugate for the binding sites. Washing of the wells using distilled water was done to remove unbound cotinine and cotinine enzyme conjugate. After addition of the substrate, the intensity of colour which was inversely proportional to the concentration of cotinine in the samples was measured and the standard curve was prepared which was relating colour intensity to the concentration of the cotinine [Table/Fig-1].

## STATISTICAL ANALYSIS

Statistical tests used for analysis were, Chi-square and t-test to find the association whereas, correlation and ANOVA were used to see the trend of cotinine levels by using SPSS version 20.0 software.

## RESULTS

Total 105 study subjects and age, parity matched control subjects were studied for cotinine levels during the period of about three months from Jan 2011-March 2011. Analysis of data showed following observations.

Maximum proportion of subjects was in the age group of 20 to 25 years (61.9%) whereas, teenagers accounted for about 24.8% in both groups. Parity wise distribution showed 62% were primipara, 24% para2, 13% para3 and 1%  $\geq$  para4. Nearly, 80% of users and 71.4% of nonusers were housewives whereas, 76.2% of users and 80% of nonusers belonged to socio-economic class III, which was statistically not significant. Significantly higher proportion of illiterates were found among users (15.2%) compared to nonusers (2.9%).

Higher proportions of users of mishri (62%) were having anaemia at the time of delivery compared to non users (19%). This difference was found statistically significant ( $\chi^2$  value=35.29,  $p=0.001$ ). Similarly

significantly higher proportion (94.3%) of users delivered before Expected Date of Delivery (EDD) compared to nonusers (81.9%) ( $\chi^2$  value=6.53,  $p=0.01$ ).

Anthropometric measurements of newborn	Mishri users (n=105)	Non Mishri users (n=105)	$\chi^2$ value	p-value
	No (%)	No. (%)		
Birth weight $\geq 2500$ gm	26 (24.8)	95 (90.5)	92.84	<0.001*
<2500gm	79 (75.2)	10 (9.5)		
Birth length $\geq 50$ cm	22 (21)	100 (95.2)	119.0	<0.001*
<50cm	83 (79)	05 (4.8)		

[Table/Fig-2]: Comparison of anthropometric measurements of newborn babies among users and nonusers of mishri. \*Highly significant.

A significantly more number of users gave birth to babies having birth weight less than 2.5 kg and length less than 50 cm compared to non users of mishri [Table/Fig-2].

It was found on analysis that, the mean cotinine level among users of mishri was  $216 \pm 101$  ng/ml whereas among nonusers it was found to be  $0.43 \pm 1.6$  ng/ml which was statistically highly significant ( $t=21.83$ ,  $p=0.001$ ).

The babies born to pregnant women using mishri were on an average 480 gm lesser in birth weight and 6.5 cm shorter in birth length compared to nonusers which was statistically highly significant.

Anthropometry of newborn babies	Mishri users (n=105)		
	Mean $\pm$ SD	r-value	p-value
Birth weight (in kg)	2.28 $\pm$ 0.23	-0.441	<0.001*
Birth length (in cm)	45 $\pm$ 3.9	-0.416	<0.001*

[Table/Fig-3]: Correlation of cotinine level in blood among users of mishri and anthropometry of newborn babies. \*Highly significant.

Cotinine levels ( $215.8 \pm 101$ ) among users were found significantly negatively correlated with anthropometric measurements of newborn babies like birth weight of baby ( $2.28 \pm 0.23$ , -0.441) and length of baby at birth ( $45 \pm 3.9$ , -0.416) [Table/Fig-3].

With increase in duration of use of mishri there was an increase in cotinine levels [Table/Fig-4]. On application of ANOVA this increasing trend was found significant. The level of cotinine was found nearly 70 ng/ml higher among those women who were using mishri since five years than those who were using it since one year. Similarly, significant increasing trend of cotinine levels was seen with increasing frequency of use, duration kept in mouth and amount of mishri used each time.

Significantly decreasing trend was seen in mean birth weight with increasing number of years of use of mishri, increasing frequency of use per day, increasing quantity of use each time and increasing duration of time kept in mouth. Whereas, such decreasing trend was observed with mean length at birth with increasing duration of use in years and increase in quantity of use each time, there was no significant trend observed with frequency of use per day and duration kept in mouth.

Subjects were grouped according to cotinine values as shown in [Table/Fig-5] with a class interval of 100 ng/dl and compared with mean values of birth weight and birth length of newborns. A significantly decreasing trend of mean birth weights and mean birth lengths at birth was observed with increase in mean group-values of cotinine. Thus a negative correlation was seen between serum cotinine levels and the mean birth weight and the mean birth length [Table/Fig-5].

[Table/Fig-6] shows a clear trend of mean increase in years of use of tobacco, frequency per day, amount per time and duration kept in mouth according to increasing levels of cotinine and also decrease in mean birth weight and birth length compared to controls.

On application of linear logistic regression to the pattern of use of mishri and cotinine levels, it showed that the amount of use of mishri each time significantly modified the levels of cotinine as compared to the years of use, the frequency per day and the duration of time kept in the mouth.

Practices of use of mishri	No.	%	Mean Cotinine level (ng/ml)±SD	Mean Birth weight Mean±SD	Mean Length at birth Mean±SD
<b>Tobacco use since (in years)</b>					
1 year	40	38.1	169.30±85.4	2.3±0.18	46±3.62
2 year	33	31.4	254.50±113.4	2.2±0.22	44±3.74
3 year	16	15.2	219.37±91.6	2.2±0.19	45±3.6
4 year	07	6.7	259.17±72.99	2.1±0.31	42±5.1
5 year	09	8.6	240.63±82.21	2.3±0.31	46±3.6
F value			4.260	3.401	3.140
p-value			0.003*	0.012*	0.018*
<b>Frequency of use per day</b>					
1 time	59	56.2	168.82±73.25	2.3±0.19	46±4.05
2 time	44	41.9	273.67±101.95	2.2±0.26	45±3.63
3 time	02	1.9	329.00±43.55	2.1±0.07	44±2.82
F value			20.34	2.933	2.322
p-value			<0.001*	0.058	0.103
<b>Quantity of use each time</b>					
<100 mg	03	2.9	63.20±18.66	2.2±0.03	44±2.12
100 mg -	51	48.6	143.44±52.70	2.3±0.19	46±3.63
200 mg -	36	34.3	259.61±34.38	2.2±0.24	45±3.90
300 mg +	15	14.3	381.94±74.63	2.1±0.22	42±3.38
F value			103.2	6.852	5.625
p-value			<0.001*	<0.001*	0.001*
<b>Duration kept in mouth in minutes</b>					
Up to 5 min	11	10.5	140.84±72.02	2.4±0.17	48±3.52
Up to 10 min	72	68.6	206.56±87.02	2.2±0.20	45±3.9
Up to ≥15 min	22	21	286.6±126.61	2.1±0.28	44±3.8
F value			6.367	4.086	2.076
p-value			0.001*	0.009*	0.108

**[Table/Fig-4]:** ANOVA test between practices of use of mishri and cotinine levels, birth weight and length at birth. \* Highly significant.

Cotinine levels (ng/ml)	No.	%	Cotinine levels (ng/ml) Mean±SD	Mean Birth weight Mean±SD	Mean Birth Length Mean±SD
Control Subjects	105	100	0.43±1.65	2.76±0.29	52±2.00
Study subjects < 100 ng/ml	17	16.2	75.35±14.98	2.45±0.11	49±2.66
100 – 199.999	28	26.7	157.09±30.47	2.34±0.20	46±3.90
200 – 299.999	43	41	246.20±32.71	2.24±0.23	45±3.60
300 +	17	16.2	376.11±71.28	2.10±0.22	43±3.36
F value =			197.42	9.375	9.088
p-value =			<0.001*	<0.001*	<0.001*

**[Table/Fig-5]:** ANOVA test between levels of cotinine and birth weight and length at birth. \*Highly significant.

## DISCUSSION

Present study shows that majority of users are in the age groups of 20-25 years, primipara, housewives, illiterates and anaemic at the time of delivery. Significant proportion of mishri users are found to be delivering before EDD, giving birth to a high proportion of LBW and shorter length at birth which has shown negative correlation with cotinine levels found in maternal blood at the time of delivery.

This study has also found a decrease in the length of the newborn (<50 cm) at birth and the proportion has been significantly higher

Cotinine levels	No.	Yrs of use	Frequency per day	Amount per time	Duration kept in mouth	Mean reduction in birth weight	Mean reduction in birth length
< 100 ng/ml	17	1.5	1.1	107 gm	3.5-5 min	310 gm	3 cm
100 – 199.99	28	1.8	1.1	158 gm	5-10 min	420 gm	6 cm
200 – 299.99	43	2.4	1.5	235 gm	6-11 min	520 gm	7 cm
300 +	17	2.5	1.9	335 gm	7-12 min	660 gm	9 cm

**[Table/Fig-6]:** Cotinine level wise pattern of tobacco use and adverse effect on Birth weight and birth length. \*Highly significant.

among users of mishri (82.9%) compared to nonusers of mishri (1.9%). Mean length of newborn among users (43 cm) was 5 cm less as compared to nonusers of mishri (52 cm) (p=.000) in the present study. No studies in India on smokeless tobacco use and decrease in birth length of the newborn are available to compare present findings. [Table/Fig-7] demonstrates comparison of present study findings with that of previous studies.

	Present study	Wang X et al., [13]	El-mohandes AAE et al., [14]	Haddow JE et al., [15]	Chang Qing Li et al., [16]	Bardy AH [17]
Birth weight	480 gm Lighter	59 gm Lighter	88 gm lighter	441 gm lighter	241 gm lighter	188 gm lighter
Length at birth	6.5 cm Shorter	0.25 cm shorter	-	-	-	10mm shorter

**[Table/Fig-7]:** Comparison of present study findings with other studies [13-17].

Wang X et al., in their prospective cohort study have found an existence of a significant inverse exposure-response relationship between cotinine concentration in maternal urine and infant size at birth [13]. For the entire gestation, a 1000 ng increase in mean urine cotinine concentration has been associated with a 59±9 gm reduction in birth weight, a 0.25±0.05 cm reduction in length, and a 0.12±0.03 cm reduction in head circumference, respectively. There are no studies available to compare the findings of cotinine level and birth weight among smokeless tobacco users in India.

El-mohandes AAE et al., studied the association between Salivary Cotinine Levels (SCLs) of pregnant women and its outcomes among black smokers [14]. The birth weights have been significantly lower for infants born to mothers with baseline SCLs. In linear regression analyses adjusting for sociodemographic and medical factors, SCLs of 20 ng/mL are associated with a reduction in birth weight of 88 gm when SCLs are measured at baseline (p=0.042) and 205 gm when SCLs are measured immediately before delivery (p=0.001). Gestational age has not seen to be affected significantly at any SCL, regardless of when SCLs are measured.

Haddow JE et al., in their study have found that smokers of ≤25 cigarettes per day, representing the 2.7% of women with the greatest cigarette consumption, have infants 289 gm lighter than the 68% of women who are non-smokers [15]. Women with serum cotinine levels in the top 2.7% (≤284 ng/ml) had infants 441 gm lighter than the 68% of women with the lower cotinine levels (≤24 ng/ml). Their results strengthen the evidence linking smoking (use of tobacco) with low birth weight and also demonstrate that cotinine can be satisfactorily used to assess and monitor cigarette smoking in pregnancy.

Chang Qing Li et al., in their study have found that those mothers who could reduce smoking among white race with initial cotinine levels greater than 100 ng/ml their infants are 241gm heavier than did white raced mothers who have continued to smoke [16].

Bardy AH et al., have observed that tobacco exposure has been associated with shorter gestational age, reduced birth weight and shorter crown-heel length of the newborns [17]. After correction for parity, gender and gestational age, the exposed newborns are on



average 188 gm (95% confidence interval (CI) 123–253 g) lighter and 10 mm (95% CI 7–13 mm) shorter than the nonexposed newborns. Cotinine level of 1 µg/ml of cotinine in maternal serum results in to a mean decrease of 1.29 gm (95% CI 0.55–2.02 gm) in birth weight and in a mean decrease of 0.059 mm (95% CI 0.035–0.083 mm) in birth length. Maternal cotinine concentrations have better explained the neonatal findings than the reported smoking habits.

Gupta PC et al., comment that the habit of tobacco abuse is developed mainly from peer pressure, friends and elders for fun [4]. The determinants of tobacco use among the youth are first of all, socio-demographic factors such as gender, state and region and rural versus urban residence and then factors affecting social norms like family influence and tobacco use by friends; exposure to advertisements in the media and community; access and availability of tobacco products in the area of residence; level of awareness about the harmfulness of tobacco and availability of tobacco products to minors; tobacco control strategies and the tactics to attract the youth used by tobacco industry.

## LIMITATION

Reduced length at birth can have implication on achievement of adult height. Detailed longitudinal studies are required to find out the effect of smokeless form of tobacco use by pregnant women and the anthropometry of baby from birth to adulthood.

## CONCLUSION

Considering about 480 gm reduction in weight and 6.5 cm reduction in height of babies born to mishri users, a pro-active effort is essential to educate the women about adverse effects of tobacco in general and on the intrauterine growth of the baby in particular. De-Addiction programmes are recommended to be undertaken in those areas where use of smokeless form of tobacco among women is widely prevalent.

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