Smokeless Tobacco (Mishri) use among pregnant women: A cross sectional study at Krishna hospital, Karad

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Abstract
Aim: This study also includes estimation of Cotinine, a metabolite of Nicotine in maternal blood using ELISA technique, which was not yet studied so far with respect to smokeless Tobacco.

Material and Method: Detailed history was taken from the subjects & from the case records as per the Proforma (Annexure I) on the day of delivery, which included personal details, diet history, obstetric history (present & past), history of parturition, anthropometric measurements of new born baby among users and non-users of Mishri. The detail of duration, frequency & amount of use as well as duration for which the Mishri was kept in mouth was collected [14]. Maternal blood (1ml) of study subjects and controls were 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 and then the levels of cotinine in serum were recorded in the respective proforma of respective participants (study subjects & controls) in nanogram per millimeter (ng/ml). All the recorded data of users & nonusers of smokeless tobacco (Mishri) were entered into the Microsoft excel sheet and appropriate statistical tests were applied to analyze the final data under guidance of the Statistician & Postgraduate Guide.

Result: Among users of Mishri no significant difference in mean levels of iron in diet was found when compared between those having Anemia & not having Anemia. Also no significant correlation was seen between hemoglobin levels and dietary iron of subjects using Mishri (r=0.047, p=4.53) whereas a significant correlation was seen among nonusers of Mishri (r=0.129, p=0.05).

Conclusion: With increasing duration it appears more and more tobacco (Mishri) is being consumed resulting in higher Cotinine levels and the adverse effects of tobacco on pregnant women and baby are accentuated.

Keywords: Cotinine, ELISA technique

Introduction
The term ‘Smokeless tobacco’ is used to describe tobacco that is consumed in un-burnt form. Smokeless tobacco can be used orally or nasally [1-3]. South East Asia is a major producer and net exporter of tobacco. With roughly 10% of the world’s smokers, India is the second largest consumer of tobacco [4] second only to China and largest producer of tobacco in the world (next to China, Brazil). [5, 6] Over one-third of tobacco consumed regionally is smokeless. Traditional forms like betel quid, tobacco with lime and tobacco tooth powder are commonly used and the use of new products is increasing, not only among men but also among children, teenagers, women of reproductive age [7].

Cotinine, a Nicotine metabolite, is used to quantify exposure to tobacco, which readily gains access to fetal circulation. Fetal Cotinine concentration in pregnant smokers is on an average 90% of maternal values throughout gestation and these values remain higher in fetal circulation for longer period [8]. Cotinine was invariably found in coelomic, amniotic and fetal serum when maternal serum Cotinine levels exceeded 25ng/ml [9]. A general impression prevails that ill effects of tobacco are related to smoking but the effects of smokeless form of tobacco consumption are underestimated.

Considering high proportion of low birth weight babies’ study of this aspect becomes important to reduce the morbidity associated with tobacco consumption during pregnancy. This study also includes estimation of Cotinine, a metabolite of Nicotine in maternal blood using ELISA technique, which was not yet studied so far with respect to smokeless Tobacco.
Also it gives us a platform to study the Dose response relationship between use of Smokeless tobacco during pregnancy and associated complications.

**Material and Method**

The following study was conducted as a Hospital based Cross sectional study at Krishna Hospital, Karad (Satara district, Maharashtra) after taking clearance from the Protocol committee and the Institutional Ethical committee. This study was planned to conduct for the period of two years, which included standardization of the method of estimation of Cotinine in blood, data collection, compilation, analysis by using appropriate statistical tests and report writing.

Study included the pregnant women who were delivered at Krishna Hospital, Karad during the period of 6months that is from 01st January to 30th June 2011. 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 are included as Study Controls after doing matching for Age and Parity\[13\].

All pregnant women who delivered at Krishna Hospital, Karad were enrolled the study subjects as well as study controls were selected as per our inclusion & exclusion criteria. Informed consent was taken from all the subjects. Detailed history was taken from the subjects & from the case records as per the Proforma (Annexure I) on the day of delivery, which included personal details, diet history, obstetric history (present & past), history of parturition, anthropometric measurements of new born baby among users and non-users of Mishri. The detail of duration, frequency & amount of use as well as duration for which the Mishri was kept in mouth was collected. \[14\] A representative sample of amount used each time was obtained from each subject and then weighed by using standard electronic weighing machine with gm discount in Biochemistry laboratory. Maternal blood (1ml) of study subjects and controls were collected in a plain bulb within 24hours 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 and then the levels of cotinine in serum were recorded in the respective proforma of respective participants (study subjects & controls) in nanogram per millimeter (ng/ml). All the recorded data of users & nonusers of smokeless tobacco (Mishri) were entered into the Microsoft excel sheet and appropriate statistical tests were applied to analyze the final data under guidance of the Statistician & Postgraduate Guide \[12\].

**Results**

During the period of six months (data collection) that is from 01st January 2011 to 30th June 2011, 2140 deliveries took place at Krishna Hospital, Karad. Among these deliveries those pregnant women who were using Smokeless Tobacco were 270. Out of these the pregnant women who were using Mishri during Pregnancy for minimum 6months of period (those who were fulfilling our inclusion criteria) were 258. Whereas 12 subjects were excluded because 1 mishri user had Twin delivery. 3 were using chewing tobacco other than mishri and 8 were using only chewing tobacco. The 258 women who were using Mishri during pregnancy were our Study subjects. The Proportion of use of Smokeless Tobacco (Mishri) by pregnant women during pregnancy among Hospital deliveries is12%.

Similar number (258) of Control subjects fulfilling inclusion criteria were selected from the consecutive deliveries during the study period after matching for Age & Parity \[11\]. The first eligible woman who delivered after the delivery of a woman from study group was selected for control group. Among the matched Study & Control subjects, around 58% were in the age group of 20 to 25 years where as 29% were teenagers and 13.2% were above 25 completed years as shown in Table I. Mean age of the subjects were found to be 22.7years with SD of 2.79. On the contrary, higher proportions of Mishri users had Anemia (HB <11g%), delivered before due date (expected date of delivery) and gave birth to babies having LBW (82%) and less birth length (83%) compared nonusers of Mishri. The proportion of birth weight in the range of 1.5 to 2kg was 11.2% whereas between 2 to 2.5 kg it was 70.5%.

Among users of Mishri no significant difference in mean levels of iron in diet was found when compared between those having Anemia & not having Anemia. Also no significant correlation was seen between hemoglobin levels and dietary iron of subjects using Mishri (r=0.047, p=4.53) whereas a significant correlation was seen among nonusers of Mishri (r=0.129, p=0.05).

**Discussion**

The proportion of pregnant women using Mishri during pregnancy among hospital deliveries in this study is 12. study conducted at Sasson general hospital Pune that 51.3% women were mishri users among hospital deliveries. Few more studies have also reported high proportion of Mishri users among Pregnant women 1991 (33.2%), 1993 (44.4%) & 1995 (42%) but Global Adult Tobacco Survey Report India 2009-10 showed prevalence of Mishri use among women (overall) in Maharashtra as 8%. By this it could be possible to comment that the proportion of pregnant women using Mishri is high which ranges from 8% to 51.3%. \[15\] This study reports that around 29% of pregnant women using Mishri are under the age of 20years (teenage group) which is comparable to other whereas Gupta P C et al. \[15\] have reported less that is 10.4% of teenage pregnant women were using Mishri.

This study has found the proportion of House wives using Mishri as 51.3% as compared to working women which was found as 45.9% and this finding is comparable to other \[11\] This study reports that nearly half proportion of users belongs to Karad taluk in which the hospital is situated whereas half proportion belongs to outside the Karad taluk (nearby places). \[10\] This equal distribution of users in two places is probably due to more prevalence of use of Mishri throughout the Maharashtra. This study has also found more number of subjects using Mishri among class III (77.9%) followed by class IV (20.5%) according to Modified B G Prasad Classification which is comparable to Gupta et al 15 study (60.5% Fby 39.5%), no single person from Class I has been using Mishri among both studies. Other studies have mentioned 35.1% in lower class (Pardeshi et al 69).

In present study proportion of women consuming adequate diet (>2200kcal/day energy & >65gm/day protein) among users is comparable with nonusers (58% vs. 52%) (p=0.18) whereas other studies have shown nearly equal results. This relation between tobacco & iron metabolism explain why in spite of sufficient iron in diet there is a high proportion of...
Anemia among users of mishri compared to nonusers of mishri. The proportions of complications associated with Pregnancy like Oligohydramnois, Fetal distress, Pregnancy induced Hypertension has been less overall but more among Mishri users than Mishri nonuser. Statistical significance is observed for Oligohydramnios & Fetal distress but not for PIH & past history of abortion. This is comparable with findings who have reported significant difference in proportions of occurrences of fetal distress & PIH. Gupta P C et al 15 have also found nearly around 3% of occurrence of PIH among both the groups. The molecular reasons are yet to be studied in depth among smokeless tobacco users.

This study found proportion of newborns having Low Birth Weight as 81.7% among users as compared to nonusers (6.2%), p=0.000 which has been very high compared to other studies like Gupta P C et al 15 have also reported a high proportions of LBW among mishri users of 19.3% vs. 9% & 28.6% vs. 19.9% among users and nonusers of Mishri respectively. Mean Birth weight of babies born to Mishri users (2.2kg) was 600gms lesser compared to nonusers of Mishri (2.8) in the present study 400gms & Gupta P C et al 15 189gms. Gupta P C et al 15 also gave a crude Relative Risk of 1.4 (95%CI=1.1 to 1.9) for LBW (RR=8; 4-14 in present study) & Krishnamurthy S et al 47 reported odds ratio for LBW of 3.2; confidence Interval 1.5 – 6.9; p<.001. This study has also found decrease in Length of newborn (<50cm) at birth & the proportion has been significantly higher among users of Mishri (82.9%) compared to nonusers of Mishri (1.9%). Mean Length of newborn among users (43cm) was 5cm less among users as compared to nonusers of Mishri (52cm) (p=.000) in the present study. No studies in India on smokeless tobacco use & decrease in Birth length of the newborn are available found to compare present finding. This study also reports that higher proportions of other family members using one or other tobacco products are Husbands & Mother-in-law and also it is the friend/neighbors/coworkers that will influence them to start using Mishri.

Conclusion
In all probability new users of Mishri are getting habituated to its use and hence if proactive efforts are made for de-addiction, they are likely to give up the habit of Mishri use more easily than the long users. With increasing duration it appears more and more tobacco (Mishri) is being consumed resulting in higher Cotinine levels and the adverse effects of tobacco on pregnant women and baby are accentuated.

Conflict of Interest: No conflict of interest

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References
2. Background - Recommendation on Smokeless Tobacco Products Scientific Advisory Committee on Tobacco Products Regulation, I-9.