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Effect of Chronic Consumption of Smokeless Tobacco (Snuff) on Liver Enzymes of Males in Maiduguri, Northeast Nigeria

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Abstract

Smokeless tobacco snuff consumption is as dangerous as cigarette smoking. It is now considered a significant source of morbidity and mortality owing to the effect of the numerous chemical constituents. Thus, this study was designed to investigate the effect of chronic consumption of Smokeless Tobacco (Snuff) on Liver Enzymes of males in Maiduguri and its environs, Northeast Nigeria. In this study we recruited 109 individuals who were smokeless tobacco snuffers and 97 healthy controls. It was a prospective case control study performed in adult males (30-50 years and 23 – 48 years for case and control respectively) with mean age of the case and control subjects of 37.12 ± 10.21and 33.64 ± 3.29 respectively. Serum Alanine amino transferase (ALT), Serum Aspartate amino transferase (AST) and serum Alkaline phosphatase (ALP) were estimated using kinetic methods by Cobas C311(Roche/Hitachi) chemistry auto analyzer. The estimated Serum AST, ALT, and ALP, levels were compared using un-paired student's t test between the two groups. Also, the liver enzyme levels were evaluated based on the duration of the smokeless tobacco consumption and correlation was determined. The age range of the study subjects was between 30 and 50 years and the mean age of the Case and control subjects were 37.12 ± 10.21and 33.64 ± 3.29 respectively. The mean(average) of the duration of tobacco snuff intake was 10.52 ± 7.02 years. The mean Serum level of AST, ALT, and ALP of tobacco snuffers were found to be higher (15.45 ± 3.32, 22.00 ± 5.10 and 22.00 ± 5.10 respectively) as compared to controls (10.42 ± 2.36, 8.88 ± 3.14 and 31.14 ± 4.60 for AST, ALT and ALP respectively) and the differences were statistically significant at $p< 0.05$. Also, the serum levels of the liver enzymes (AST, ALT, and ALP) were evaluated according to duration of snuff intake and the liver enzymes were found to be higher in people who uses the tobacco snuff for 11-20 years. However, the duration of the snuff intake was correlated with the serum levels of the liver enzymes. There was strong correlation between duration of snuff intake and serum levels of AST and ALT ($r=0.648$ and $r=0.741$ respectively) and the relationship were statistically significant at $p<0.05$. But the serum level of ALP was weekly correlated with the duration of snuff intake and the relationship was not statistically significant at $p>0.05$. From the results of the current study and the literature reviewed it is evident that tobacco snuff may likely be one of the causes of several liver diseases. Therefore, our study might be helpful in creating awareness on the hazards of using smokeless tobacco products (Snuff), among our population who are using smokeless tobacco.

Keywords: effect, snuff, tobacco, enzymes, smokeless

Introduction

Tobacco which is botanically known as *Nicotiana tabacum* is a perennial herbaceous plant which is the most commonly grown of all plants in the genius *Nicotiana* (Ugbor *et al*., 2013). More than 65 species of tobacco plant are known to exist. Tobacco is a general name for any product prepared from the leaves of these plants. The plant is commercially grown in many countries where the leaves are used to process the

production of tobacco products. The products are prepared in different forms for different use such as for smoking as in cigarettes, cigars and pipes and can also be consumed as in snuff, and chewing tobacco (Rudgley and Richard, 2017). Globally, non-communicable diseases such as chronic respiratory disease, ischemic heart diseases, cancers, and diabetes are the leading causes of death and these diseases are found to be associated with tobacco use (WHO, 2008 and GATS, 2014). In middle- and low-income countries, about 38 million people die each year from this disease (WHO, 2010). In India, about 274.9 million people use tobacco out of which 163.7 million use only smokeless tobacco, 68.9 million only smokers and 42.3 million users of both smoking and smokeless as per Global Adult Tobacco Survey India (GATS) (GATS, 2014).

In Nigeria, tobacco snuff is prepared and produced locally from the tobacco leaves mixed with other substances such as potash, black pepper, alligator pepper, salt, ayaro, cloves and in most products flavors and fragrances are added. The products are commercially available and differ according to the nature and components mixtures (Shaayau and Mohammed, 2019). The consumption is on the increase in most developing countries including Nigeria. The increase in the consumption and the availability of this product (Snuff) may be related to lack of legislation against its usage and therefore promoting its widespread marketing making it available and affordable for the users. The increase in the consumption may also be related to some belief that these products have some medicinal benefits such as cure of catarrh, headache, pile, and also energy booster and a sexual enhancer thus causing potential addiction (Ugbor *et al*., 2013). Again, others use it as tradition in social gathering in unspecified quantities without the knowledge of its potential dangers to health (Musa *et al*., 2019). It is this addiction and irrational use of these products that may expose the users to numerous health risks. Tobacco products contains more than 490 toxic compounds (Bonnie *et al*., 2015). Tobacco (smoked or smokeless) contains Nicotine and other phytochemical constituents such as potent tobacco-specific carcinogen 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone), 4-methyl-nitrosamino)-4-(3-pyridyl)-butanal (NNA), and N-Nitrosonornicotine, heavy metals (Cadmium, Mercury etc.) and 23 polycyclic aromatic hydrocarbons which has been implicated with tobacco associated cancers and diseases (Ugbor *et al*., 2013, Jasmine *et al*., 2019). The major ingredient in tobacco, alkaloid nicotine is metabolized and detoxified in the liver. Nicotine inhibits antigen mediated signaling in T-cells and this block the proliferation and differentiation of lymphocytes and suppression of antibody forming cells. This leads to increased production of pro-inflammatory cytokines (IL-1, IL-6 and TNF-a) which are involved in liver cell injury (El-zayadi, 2006). Studies have shown that there is a decrease in antioxidants enzymes [hepatic Glutathione (GSH), Glutathione peroxidase (GPx), Super oxide dismutase (SOD) and catalase (CAT)] and increase lipid peroxidase (LPx) (Pramod, 2006). These factors lead to inflammation of the liver (Mitchell *et al*., 2013; Velayutharaj *et al*., 2013). However, the effect depends on the amount and duration of consumption of the smokeless snuff (Velayutharaj *et al*., 2013).

However, there is paucity of data on the effect of chronic consumption of smokeless tobacco (snuff) on liver enzymes in male consumers in this area. Hence, it is imperative to assess the liver enzymes of people who consume these products. We believe that this study will help to increase awareness about harmful effect of the toxic chemicals present in tobacco snuff products of this area.

Materials and Methods

This was a prospective case control study. A total of 206 adult males were recruited for the study out of which 109 were cases (Tobacco Snuff Users) between the ages 30 and 50 years and 97 were control between the age of 30 and 48 years. Controls were healthy subjects who have the same dietary feeding habits and who had no previous history of using tobacco in any form.

The Ethical Clearance:

Protocol was duly submitted to the Institutional Human ethics committee of Borno State Ministry of Health and approval was granted

before starting the study. All the procedure was informed to the paticipants in a language they understand and written informed written consent was obtained from the participants. Sociodemographic data was obtained using a questionnaire.

Blood sample collection

Five milliliters (5 ml) of blood specimen was collected from each subject for biochemical analysis. The blood was collected aseptically from the antecubital vein using sterile disposable 5 ml syringe and transferred into an appropriately labeled plain container and allowed to clot at room temperature. The samples were centrifuged at 4000 revolutions per minute (rpm) for 5 minutes and serum separated into another appropriately labeled sample containers(cryovial) and stored frozen until the time of analysis.

Anthropometry:

Anthropometric measurements were also taken for each subject. This includes: Height, Weight, Blood pressures(bp) both systolic and diastolic bp.

Exclusion criteria: [For both control and cases groups]:

Males <30 and >50 years of age. Patients with liver pathology (such as Viral Hepatitis, post hepatic jaundice), Systemic diseases (such as Metabolic syndrome, Sickle cell disease etc.), Kidney or Bone disorder. Males who consume alcohol in any form and those who are on traditional medicine.

Estimation of biochemical parameters

All biochemical analysis was conducted at university of Maiduguri teaching hospital, Maiduguri, Borno state Nigeria.

The following biochemical parameters were estimated based on established spectrophotometric and automated procedures, approved by the International Federation of Clinical Chemistry and Laboratory medicine (IFCC). Serum Alanine amino transferase (ALT), Serum Aspartate amino transferase (AST) and serum Alkaline phosphatase (ALP) were estimated using kinetic methods by Cobas C311(Roche/Hitachi) chemistry auto analyzer.

Test Principle

AST

AST in the sample catalyzes the transfer of an amino group between L- aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD+.

$$\text{L-Aspartate} + \text{2-oxoglutarate} \xrightarrow{\text{AST}} \text{oxaloacetate} + \text{L- glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow{\text{MDH}} \text{L-malate} + NAD^+$$

The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance.

ALT

ALT in the sample catalyzes the reaction between transfer L- alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH), to form L-lactate and NAD^+

$$\text{L-Alanine} + \text{2-oxoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{L-glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{MDH}} \text{L-lactate} + NAD^+$$

The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance.

ALP

Colorimetric assay in accordance with a standard method. In the presence of magnesium and Zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol.

$$\text{p-nitrophenyl phosphate} + H2O \xrightarrow{\text{ALP}} \text{Phosphate} + \text{p-nitrophenol}$$

The p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance.

Statistical analysis:
All the data were expressed as Mean ± SD; unpaired Students t- test was used to compare the data between the two groups of smokeless tobacco Snuffers and healthy control. A p value <0.05 was statistically considered significant for all statistical tests. SPSS 20.0 package was used for all statistical analysis.

Results
Two hundred and seven subjects were recruited for this study. One hundred and nine (109) 52.7% were case (Tobacco snuffers) and ninety-seven (97) 46.9% were control. The mean age of the Case and control subjects were 37.12 ± 10.21and 33.64 ± 3.29 respectively. Other anthropometric variables are as indicated in table1.The mean(average) of the duration of tobacco snuff intake was 10.52 ± 7.02.

The mean Serum level of AST, ALT, and ALP of tobacco snuffers were found to be higher (15.45 ± 3.32, 22.00 ± 5.10 and 22.00 ± 5.10 respectively) as compared to controls (10.42 ± 2.36, 8.88 ± 3.14 and 31.14 ± 4.60 for AST, ALT and ALP respectively) and the differences were statistically significant at p< 0.05 as shown in table 2. Also, the serum levels of the liver enzymes (AST, ALT, and ALP) were evaluated according to duration of snuff intake as indicated in table3. The liver enzymes were found to be higher in people who uses the tobacco snuff for 11-20 years. However, the duration of the snuff intake was correlated with the serum levels of the liver enzymes. There was strong correlation between duration of snuff intake and serum levels of AST and ALT (r=0.648 and r=0.741 respectively) and the relationship were statistically significant at p<0.05. But the serum level of ALP was weakly correlated with the duration of snuff intake and the relationship was not statistically significant at p<0.05 as shown in table4. This relationship is evident from the scatterplots in figures 1, 2 and 3.

Table 1. Anthropometric measurement of both Case and Control subjects at p<0.05

Parameters	Case(n=109) Mean ± SD	Control(n=97) Mean ± SD
Age (Years)	37.12 ± 10.21	33.64 ± 3.29
Weight(gms)	64.63 ± 11.03	62.69 ± 11.42
Height(m)	1.71 ± 0.07	1.69 ± 0. 08
BMI(g/m^2)	22.28 ± 3.48	24.22 ± 20.38
SBP (mmHg)	132.99 ± 17.75	127.13 ± 15.48
DBP (mmHg)	84.25 ± 10.33	77.45 ± 9.61

Table 2. Mean values of Serum levels of Liver enzymes in both case and Control subjects at p<0.05

Parameters	Case(n=109) Mean ± SD	Control(n=97) Mean ± SD	P-value
AST(U/L)	15.45 ± 3.32	10.42 ± 2.36	0.002
ALT(U/L)	22.00 ± 5.10	8.88 ± 3.14	0.000

P-value < 0.05 is significant

Table 3: Mean values of Serum Enzymes according to Duration of smokeless tobacco Snuff intake

Duration in years	No of subjects (n=109)	AST (U/L) Mean ± SD	ALT (U/L) Mean ± SD	ALP (U/L) Mean ± SD
5-10	44(21.3%)	15.65 ± 2.59	21.34 ± 4.32	33.56 ± 7.06
11-20	45(21.7%)	15.56 ± 3.78	22.87 ± 5.36	33.58 ± 6.66
21-30	10(4.8%)	14.50 ± 4.01	21.10 ± 6.04	28.20 ± 8.52
31-40	10(4.8%)	15.00 ± 3.52	21.90 ± 6.24	32.80 ± 5.59

Table 4: Correlation between duration of smokeless tobacco snuff intake and ALP, ASAT and ALAT activity

Variables	r-values	p- values	Remark
Duration of snuff intake in years and AST activity (IU/L)	0.648	0.000	S
Duration of snuff intake in years and ALT activity (IU/L)	0.741	0.000	S
Duration of snuff intake in years and ALP activity (IU/L)	0.126	0.072	NS

S = Significant

NS= Not Significant

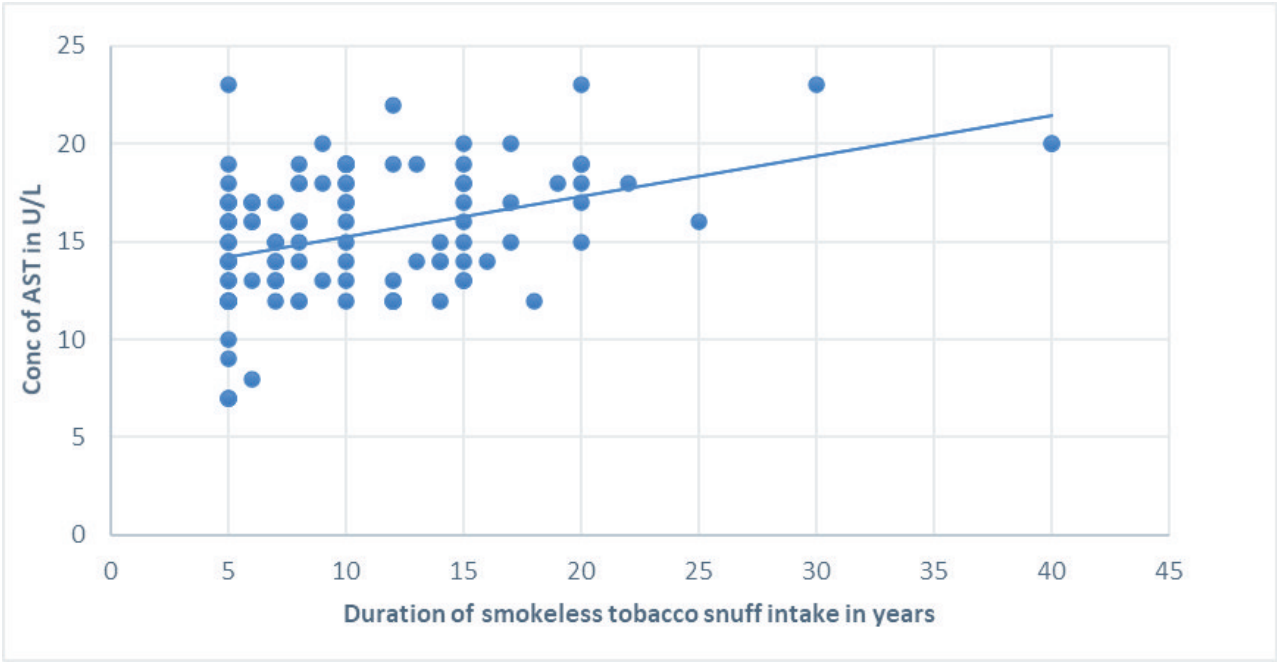

Figure 1. Scatterplot of Duration of smokeless tobacco snuff intake in years and Concentration(conc) of AST in U/L

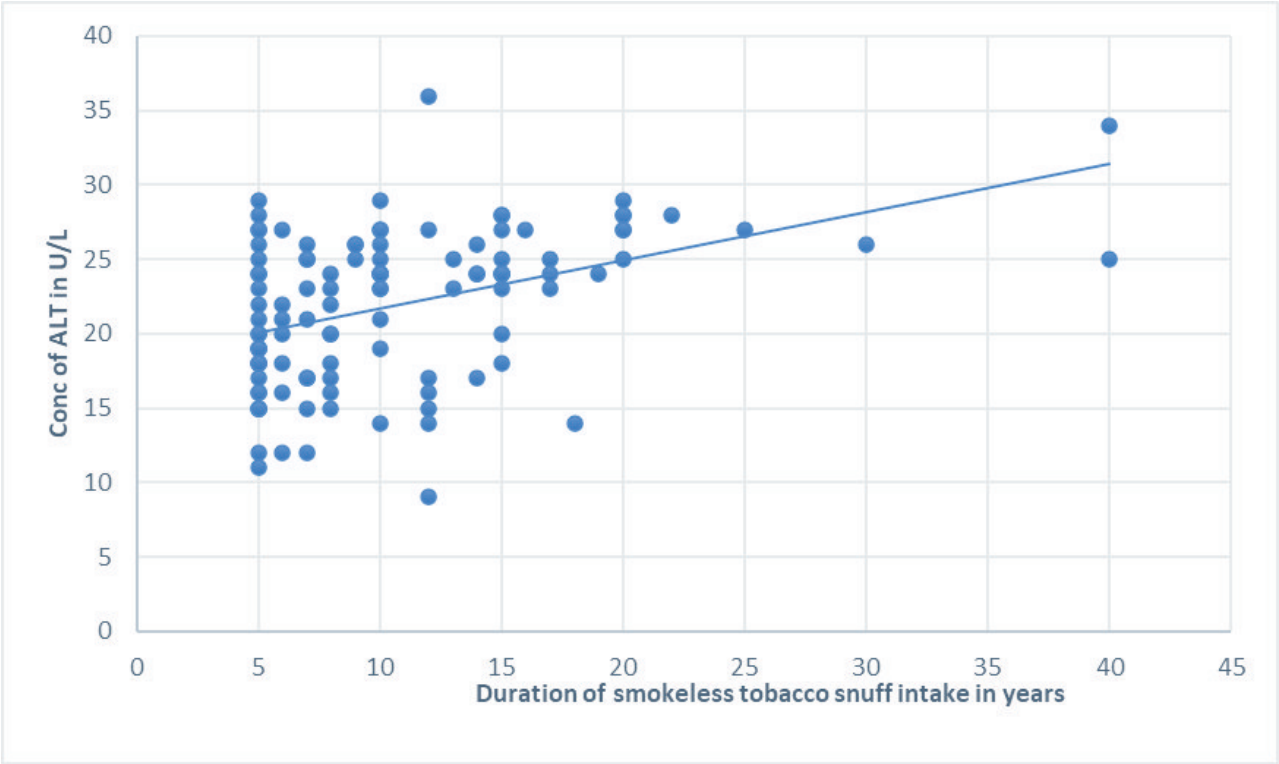

Figure 2. Scatterplot of Duration of smokeless tobacco snuff intake in years and Conc of ALT in U/L

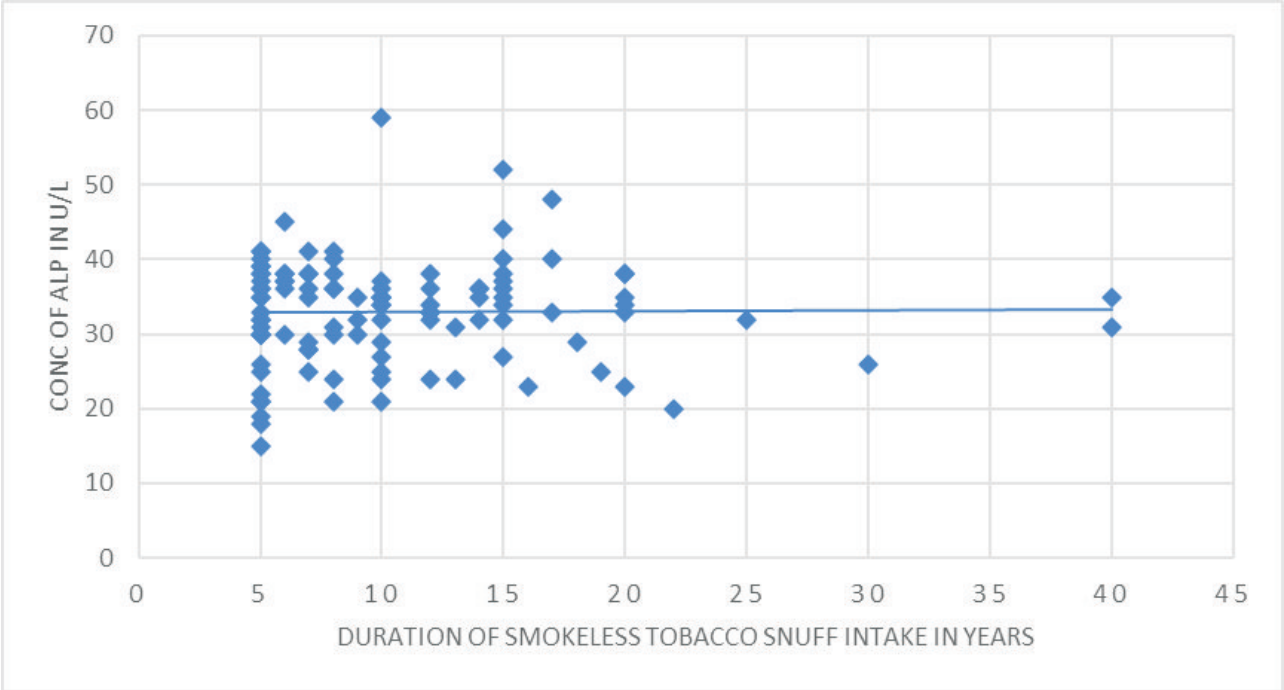

Figure 3. Scatterplot of Duration of snuff intake in years and Conc of ALP in U/L

Discussion

Tobacco whether smoked or smokeless, contains Nicotine and other phytochemical constituents such as potent tobacco-specific carcinogen 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone), 4-methyl-nitrosamino)-4-(3-pyridyl)-butanal (NNA), and N-Nitrosonornicotine, heavy metals (Cadmium, Mercury etc.) and 23 polycyclic aromatic hydrocarbons which has been implicated with tobacco associated cancers and diseases (Ugbor *et al*., 2013).

In this study we recruited 109 individuals who were smokeless tobacco snuffers and 97 healthy controls. It was a prospective case control study performed in adult males between the ages 30 - 50 years.

In this study we tried to assess the effect of tobacco snuff on the liver. We evaluated the mean serum liver enzyme levels of AST, ALT and ALP as a marker of liver damage in individuals who use tobacco snuff and compared with those of control individuals. The result showed higher levels of the liver enzymes in tobacco snuffers as compared to the control subjects and there were significant statistical differences at $p<0.05$. This finding was in agreement with the work of Velayutharaj Alwar *et. al* (2013) who reported similar finding in India. In a similar research conducted by Ugbor *et.al* (2013) in Enugu Nigeria, the authors reported similar findings on the liver enzymes AST, ALT and ALP. However, they reported that ALP shows no statistically significant increase which is contrary to our finding. In another research conducted by Shaayau and Mohammed (2019) in Sokoto Nigeria, the researchers reported their findings in Wister albino Rats similar to our findings. Our finding is also in agreement with the report of Al-Mukhaini *et al* (2017) who reported that liver enzymes increase significantly at $p<0.05$

The high level of serum enzymes observed in our study may be due to the effect of Nicotine and other phytochemical constituents present in Tobacco snuff and which has hepatocellular toxicity. These chemicals are implicated in the inhibition of antigen mediated signaling in T-Cells blocking the proliferation and differentiation of lymphocytes and suppression of antibody forming cells leading to production of other chemicals in the body which are involved in liver cell injury causing increase in the liver enzymes (Ugbor *et al* ,2013). This study also evaluated the liver enzymes according to the duration of the tobacco snuff intake. The enzyme levels were found to be higher in people who uses tobacco snuff for a period of 11-20years of intake. However, there was strong correlation between duration of tobacco snuff intake and serum levels of AST and ALT ($r=0.648$ and $r=0.741$ respectively) and the relationship were statistically significant at $p<0.05$. The serum level of ALP was weakly correlated ($r=0.126$) with the duration of tobacco snuff intake and there was no statistically significant relationship ($p=0.072$). This study therefore indicates that tobacco snuff toxicity on liver and the observed changes were duration dependent. This is in agreement with report of Ugbor *et al* (2013) that potash-tobacco dust (local tobacco snuff) is toxic to the liver and the observed changes were dose and duration dependent.

Conclusions

From the results of the current study and the literature reviewed it is evident that tobacco snuff may likely be one of the causes of several liver diseases since its production and consumption is growing rapidly and is alarmingly becoming prevalent in most part our country, Nigeria. This may be due to lack of legislation against its usage and therefore making its widespread marketing, availability, affordability and perhaps may also be due to the belief that it has some medicinal effects with consequence of potential addiction and effect on health.

Therefore, our study might be helpful in creating awareness on the hazards of using smokeless tobacco products (Snuff), among our population who are using smokeless tobacco Snuff.

Recommendations

Further Cohort study is suggested to evaluate the incidence rate of the effect of tobacco snuff consumption.

Legislation against the use of this smokeless tobacco snuff consumption similar to that of tobacco smoking should be established.

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