"Tobacco-Induced Alterations in Thyroid and Hepato-Biliary Biomarkers: A Cross-Sectional Insight"

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ABSTARCT:

Background: Tobacco consumption through smoking or chewing causes both physiological and psychological addiction which negatively impacts multiple organ systems, including the thyroid and liver. Although various biochemical alterations are associated with tobacco use, limited studies specifically examine liver function and thyroid profiles in this population. To know the correlation between tobacco exposures, liver function tests, and thyroid profiles in tobacco users.

Methods: Biochemical parameters including blood sugar (FBS, PPBS), liver enzymes (ALT, AST), bilirubin fractions, lipid profile, and thyroid hormones (FT3, FT4, TSH) were evaluated and statistically correlated in individuals with tobacco use.

Results: Significant correlations were observed among several parameters. FBS and PPBS were associated with bilirubin levels and protein markers. Total protein correlated positively with albumin, liver enzymes, and lipid parameters. Bilirubin showed strong interrelations and association with liver enzymes. FT3 and FT4 were positively linked and inversely related to TSH. Among tobacco chewers, age was negatively correlated with total cholesterol and LDL.

Conclusion: Tobacco use is significantly associated with altered thyroid and liver function profiles, with particularly strong associations observed in smokers. These findings suggest a potential risk for future hepatobiliary and thyroid disorders in chronic tobacco users.

INTRODUCTION:

Tobacco consumption continues to be a significant public health challenge worldwide, contributing to the global burden of chronic diseases. The World Health Organization reports that tobacco use is responsible for more than 8 million deaths each year, including 1.2 million from exposure to second-hand smoke (1).Approximately, 194 million people aged 15 years and older (150 million men and 44 million women) consumed some form of tobacco. Almost 79% of tobacco consumers lived in rural areas (2).The most frequent source of hazardous chemical exposure and disease caused by chemicals in people is thought to be by tobacco products. Smoking has various negative effects on organs that don't directly interact with smoke.The active component in tobacco is nicotine, which is highly addictive and promotes sustained tobacco use. The consumption of tobacco products is split into fuel and nonfuel. Fuel products include pipes, water pipes (hookah), small cigars and cigarettes. Tobacco

formulations and electronic cigarettes created for snuffing, dipping, and chewing are noncombustible tobacco products (3).

Smoking have been linked to the development of autoimmune disease.Tobacco is consumed in both smoked such as cigarettes, beedies, cigars and smokeless forms like chewing tobacco, snuff, gutkha, and both forms are prevalent in developing countries like India (4). Despite increased awareness, tobacco remains deeply embedded in cultural practices, particularly among certain socioeconomic and occupational groups.The most significant preventable risk factor for a number of malignancies, including acute myeloid leukemia, lung, esophageal, laryngeal, mouth, throat, kidney, bladder, stomach, cervix, and colorectal cancers, is smoking. (5)The number one preventable cause of mortality is tobacco use, and by 2020 it is predicted to be the third most common cause of death worldwide.(6)

Tobacco exerts harmful effects on nearly every organ of the body, through both direct exposure but also via systemic circulation of its toxic components. Nicotine and other constituents of tobacco can disrupt various biochemical pathways, contributing to oxidative stress, inflammation, and altered metabolism (7,8). These biochemical effects are not only confined to the lungs or cardiovascular system rather, they influence organs like the liver and thyroid, which do not come into direct contact with tobacco smoke (9).

The liver is the central organ for metabolism and detoxification. It is particularly susceptible to tobacco-induced toxicity. Studies have shown that tobacco use can elevate liver enzymes such as ALT, AST, and ALP, indicating hepaticdamage. Additionally, alterations in bilirubin levels and serum proteins induce inflammationor hepatocellular dysfunction (10). Chronic exposure to tobacco may exacerbate liver fibrosis, disrupt lipid metabolism, and impair hepatic clearance of hormones and toxins (11).The thyroid glandcan be affected by exposure to tobacco. Smoking has been associated with altered levels of thyroid hormonesand has been linked to increased risk of both hyperthyroidism and hypothyroidism (12).

Obese people are more likely to smoke cigarettes, making them more likely to develop nonalcoholic fatty liver disease (NAFLD) (13). In both people and lab animals, a number of tobacco smoke components have been shown to cause liver cancer. It is known that N-nitrosodimethylamine causes liver tumors in a variety of animals (14). Smoking is linked to hepatocellular carcinoma developing more quickly in people with chronic hepatitis B or C virus infection (15). Low levels of serum bilirubin, an endogenous antioxidant biomarker, may be a risk factor for illnesses linked to smoking.Particularly in regions with weak defence mechanisms including the myocardium, coronary arteries, and nervous system, serum bilirubin defends lipid membranes, albumin, and other proteins (16).

There remains a significant gap in the literature regarding the effects oftobacco on liver and thyroid functions especially among smokeless tobacco users. Only limited data is on hepatobiliary and endocrine disruptions. This study aims to bridge that gap by finding the relationship between tobacco usage and basic biochemical parameters, including liver function tests and thyroid profile.

MATERIALS AND METHODS:

It was a cross-sectional observational study. A total of 105 patients participated in the study. The study population included adult tobacco users of both types i.e., smokers and chewers, attending a tertiary care hospital. Participants were selected based on:

INCLUSION CRITERIA:
Individuals consuming tobacco in any form between 18 to 60 years.Tobacco consumers who were cigarette smokers (≥5 cigarette per day) and had prior experience with the use of oral snuff and chewing tobacco.

EXCLUSION CRITERIA:
Patients with Type II diabetes mellitus, Respiratory disease, Cardiovascular disease, Cancer, Pregnancy and Other chronic illness.

DATA COLLECTION:

Demographic data such as age, gender, duration and type of tobacco use were obtained.

BIOCHEMICAL PARAMETERS:

Blood samples were collected by a trained researcher after 12 hours of overnight fasting, following standard phlebotomy procedures to ensure consistency and minimize pre-analytical variability.

Fasting plasma glucose was measured using the glucose oxidase-peroxidase (GOD-POD) method, in which glucose is oxidized by glucose oxidase to produce hydrogen peroxide. This hydrogen peroxide then reacts with a chromogen in the presence of peroxidase to form a colored compound, the intensity of which is measured spectrophotometrically and is proportional to glucose concentration. riacylglycerols were quantified enzymatically through the action of lipoprotein lipase, which hydrolyzes triacylglycerols to release free fatty acids. These fatty acids undergo further enzymatic reactions resulting in the formation of a measurable colored product proportional to triacylglycerol concentration.

Total cholesterol was measured by first hydrolyzing cholesterol esters with cholesterol esterase to free cholesterol, which was then oxidized by cholesterol oxidase, producing hydrogen peroxide. The hydrogen peroxide subsequently reacts with phenol and 4-aminoantipyrine to form a quinoneimine dye, which is read spectrophotometrically at 510 nm. High-density lipoprotein cholesterol was determined directly from fasting serum using a polyanion precipitation method that removes non-HDL lipoproteins, allowing the measurement of HDL cholesterol in the supernatant. Low-density lipoprotein cholesterol was calculated using the Friedewald formula, which derives LDL levels from total cholesterol, HDL cholesterol, and triglyceride values.

Total serum protein was estimated by the Biuret method, where peptide bonds react with copper ions in an alkaline solution to form a violet complex measured spectrophotometrically. Albumin levels were determined based on its binding with Bromocresol Green dye at pH 4.2, causing a color change measured between 540 and 630 nm, with maximum absorbance at 625 nm; globulin levels were calculated by subtracting albumin from total protein. Aspartate aminotransferase activity was measured by monitoring the conversion of L-aspartate and 2-oxoglutarate to L-glutamate and oxaloacetate, with oxaloacetate further reduced to L-malate by malate dehydrogenase using NADH as a cofactor. The decrease in absorbance due to NADH oxidation was used to quantify enzyme activity. Similarly, alanine aminotransferase activity was determined through the conversion of L-alanine and 2-oxoglutarate to L-glutamate and pyruvate, followed by pyruvate reduction to D-lactate by lactate dehydrogenase, with NADH oxidation monitored spectrophotometrically.

Alkaline phosphatase activity was assessed by measuring the hydrolysis of p-nitrophenyl phosphate to p-nitrophenol and phosphate, where the liberated p-nitrophenol produces a yellow color proportional to enzyme activity. Uric acid concentration was measured by its enzymatic oxidation to allantoin using uricase. This reaction produces hydrogen peroxide, which subsequently reacts with 4-aminoantipyrine and 2,4,6-tribromo-3-hydroxybenzoic acid (TBHBA) to form a quinoneimine dye. The intensity of the colored quinoneimine product, measured spectrophotometrically, is proportional to the uric acid concentration in the sample. Creatinine levels were estimated using the alkaline picrate method, where creatinine reacts with picric acid to form an orange-red creatinine-picrate complex. The absorbance change at specific time intervals during the formation of this complex is directly proportional to the creatinine concentration.

Total bilirubin was determined using the Jendrassik and Gróf method, which involves coupling bilirubin with diazotized sulfanilic acid to form an azo dye. In this reaction, water-soluble bilirubin glucuronides react directly, while free (indirect) bilirubin requires an accelerator such as caffeine, sodium benzoate, and sodium acetate. The resulting blue azobilirubin formed in alkaline Fehling's solution is measured photometrically at 578 nm. Direct bilirubin was quantified through a photometric test using 2,4-dichloroaniline (DCA), where direct bilirubin reacts in acidic solution to form a red azo compound measured at 546 nm. Indirect bilirubin was calculated by subtracting the direct bilirubin value from the total bilirubin concentration.

RESULTS:

Descriptive statistics were used to summarize demographic and biochemical data. Pearson's correlation coefficient was applied to assess relationships between tobacco exposure and biochemical parameters. Data analysis was performed using SPSS version 2.0.

Table 1 - Correlation Coefficient between Liver Function, Lipid Profile and Other Biochemical Parameters

VARIABLES		r -value	p -value
FBS	TSH	0.224	0.021#
	Creatinine	0.292	0.003*
Uric Acid	LDL	0.216	0.027#
	FT4	0.200	0.050#
TC	Total Protein	0.260	0.007*
LDL	Total Protein	0.275	0.005*
Age	Uric Acid	-0.217	0.026#
Weight	Uric Acid	0.277	0.020*

FBS - Fasting blood sugar, TSH – Thyroid stimulating hormone, TC - Total cholesterol, LDL - Low density lipoprotein, FT4 – free thyroxine.

#statistically significant p value <0.05 *statistically significant p value <0.01

Table 2 depicts the correlation of biochemical parameters as observed in tobacco consumers. FBS had been strongly associated with TSH and creatinine with p - values <0.05, <0.01 and also shown in graph figure. 1 and 2. Uric acid was effectively linked with LDL and FT4 with p - values <0.05 shown in fig 3. In tobacco chewers age was negatively interconnected with uric acid with the p - value of <0.05 shown in fig 4 and weight was positively connected with uric acid with the p - value of <0.05.

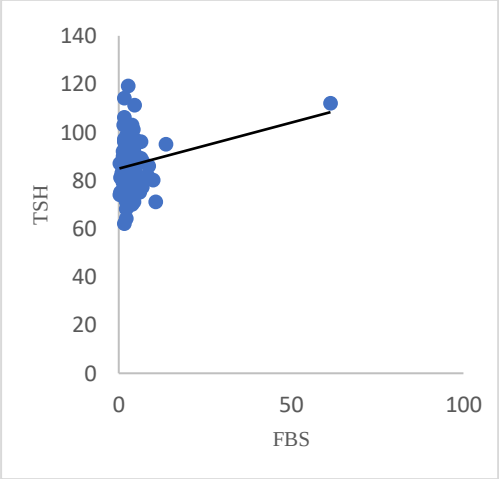


Fig. 1 – Correlation between Fasting Blood Sugar Levels and Thyroid Stimulating Hormone

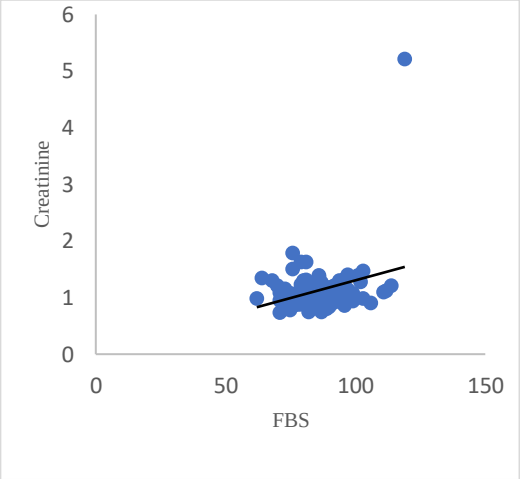
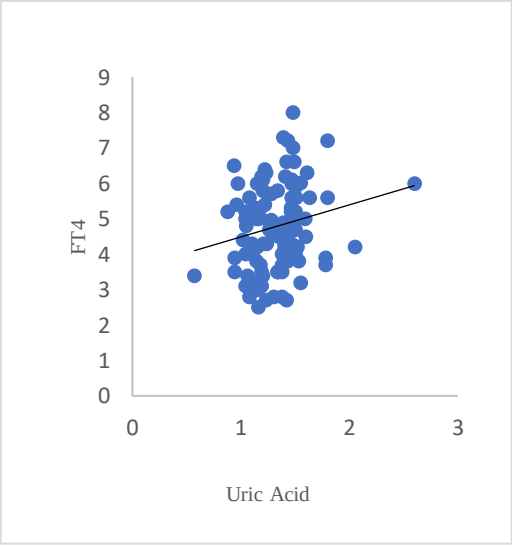


Fig. 2 – Correlation between Fasting Blood Sugar levels and Creatinine



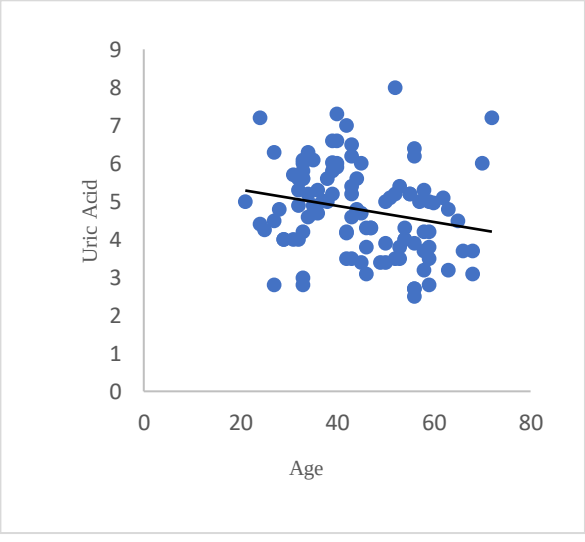


Fig.3 - Correlation between FT4 and Uric acid levels

Fig. 4 – Correlation between age and Uric acid levels

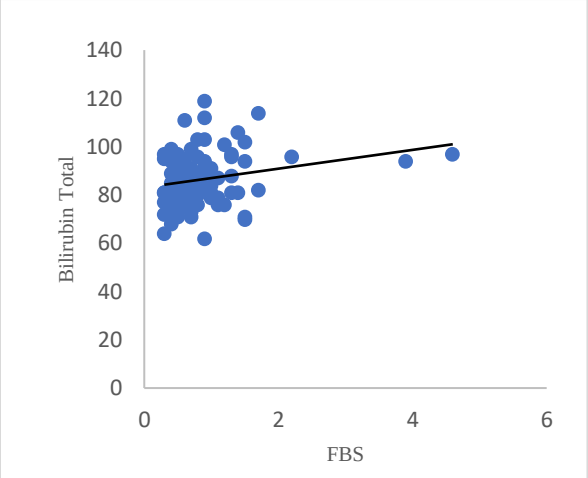


Fig.5 - Correlation between Bilirubin and Fasting Blood Sugar Levels

Fasting Blood Sugar (FBS) and Post Prandial Blood Sugar (PPBS) was associated with bilirubin fractions and protein markers, particularly FBS was positively correlated with total and indirect bilirubin level which can be understand as high blood glucose levels might be linked to increased bilirubin that is used as a marker of liver functioning. Conversely, Fasting Blood Sugar levels showed negative association with globulin, total, and direct bilirubin. This means there is a complex interplay between glucose metabolism and liver protein synthesis in tobacco users. PPBS were moderately correlated with the albumin/globulin (A/G) ratio and glycatedhemoglobin (HbA1c), indicating the potential influence of postprandial glucose spikes on liver protein balance and long-term glycemic control.

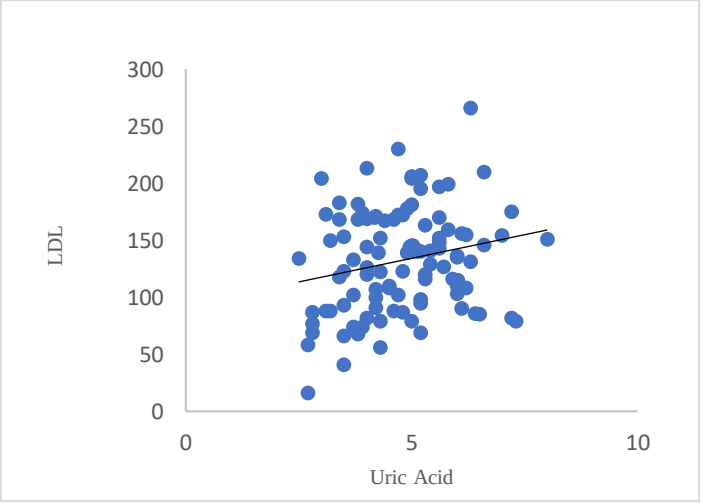


Fig. 6 - Correlation between LDL Cholesterol and Uric Acid

Total protein levels increased along with albumin, globulin, ALT, total cholesterol, and LDL cholesterol, but decreased as the A/G ratio went up. Globulin was strongly linked with both total and direct bilirubin but showed an opposite trend with the A/G ratio. The A/G ratio, in turn, dropped as total and direct bilirubin rose. Total bilirubin was closely connected with direct and indirect bilirubin, as well as AST, and direct bilirubin was strongly related to indirect bilirubin. Indirect bilirubin also increased with AST, while ALT and AST were strongly linked with each other.

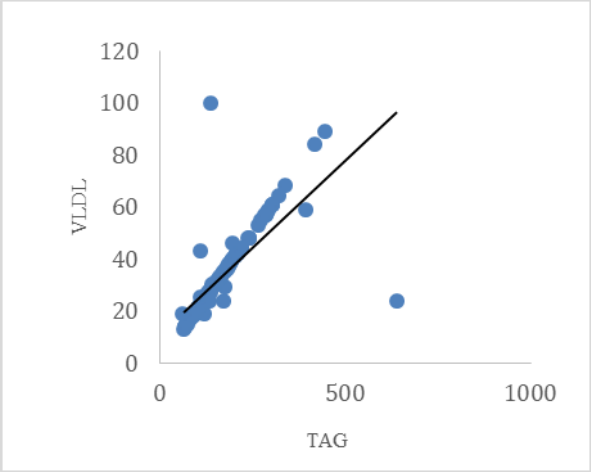


Fig. 7 - Association between VLDL and Triglycerides

The lipid profile also showed significant correlations. Total cholesterol had a strong positive relationship with both HDL and LDL cholesterol, while triglycerides were strongly linked with VLDL. HDL also showed a moderate correlation with LDL. Interestingly, in tobacco chewers, age was negatively correlated with TC and LDL, implying that the impact of tobacco on lipid parameters might vary with age or duration of exposure.

Within the thyroid profile, FT3 was positively correlated with FT4 but negatively associated with TSH. FT4 was also inversely related to TSH. This pattern reflects the normal physiological feedback mechanism but may also indicate subtle thyroid dysfunction or altered hormonal regulation among tobacco users.

DISCUSSION:

Magdalena Wronka et al., reported that smoking cigarettes can lead to disease via a process involving elevated levels of systemic inflammation and free radical generation. Smokers are more likely to develop T2DM than non-smokers (15). Our study supported as the previous literature at which fasting glucose has been strongly correlated with total and indirect bilirubin, whereas PBS has been negatively associated with globulin, total and direct bilirubin and moderately correlated with A/G ratio and HbA1c. Chang et al., reported that Due to the significantly lower prevalence in males compared to women, the majority of studies found that the inverse link between current smoking and thyroid cancer was statistically significant in women but not in men.

Smoking and thyroid cancer are linked by mechanisms that are not fully understood, but several theories have been proposed. Cigarette smoking has been associated with changes in thyroid function tests, including decreased thyroid-stimulating hormone (TSH) levels and

increased circulating thyroid hormones (16). This is relevant because higher TSH levels have been associated with increased risk and more advanced stages of thyroid cancer (17). Some researchers suggest that the apparent protective effect of smoking on thyroid cancer may be due to these lower TSH levels and a generally lower body mass index (BMI) observed in smokers, both of which are linked to a lower risk of thyroid cancer (18, 19).

In our present study, TSH and FT4 have been strongly associated with A/G ratio. Bjørn O. Asvold et al., reported that after stopping smoking, it was observed that thyrotropin levels increased gradually. About 10 to 20 years later, the levels of thyrotropin in former smokers were similar to those of never smokers. Additionally, the prevalence of hypothyroidism or hyperthyroidism was the same in long-term quitters as it was in never-smokers. Smokers had a decreased prevalence of high thyrotropin levels, according to population-based studies' findings, which are compatible with our conclusion regarding subclinical hypothyroidism. Moreover, an earlier research revealed either no link or an increased incidence of hypothyroidism among smokers; they discovered that current smoking was associated with a decreased prevalence of overt hypothyroidism (20).

Our current study represents freeT3 has positively linked with free T4, while negatively linked with TSH whereas freeT4 had negatively associated with TSH. Some past studies concluded that, Smoking cessation was linked to a decreased risk of Graves disease than continued smoking. In Graves' disease and nontoxic goitre, men appeared to be protected against the harmful consequences of smoking (21). However, prior research has shown a direct relationship between thyrotropin levels and body mass, and it is also generally known that quitting smoking has a weight-gaining effect (22). Present study shows that age of study subjects was negatively interconnected with uric acid and weight is positively associated with uric acid.

In tobacco chewers persons age was negatively interconnected with TC and LDL. According to Oliveira et al the 15-day-old nicotine offspring had reduced serum-free triiodothyronine (FT3) and thyroxine (FT4) with greater TSH. In a previous study, smoking either has no effect on adult thyroid function or has a modest pro-thyroid effect, resulting in minor, thyrotrophin-independent increases in thyroid function, most frequently small increases in blood triiodothyronine concentrations.

In our present study the correlation of biochemical parameters as observed in tobacco consumers such as total cholesterol had strong positive interrelation with HDL and LDL. TAG has been effectively associated with VLDL. HDL had moderate relationship with LDL. FT3 has been positively linked with thyroxine, while negatively linked with TSH. FT4 had negatively associated with TSH. Smoking alters the metabolism of several medicines, primarily by affecting the liver metabolism. Yuan-Chin Amy Lee et al., reported that the link between cigarette smoking and the chance of developing liver cancer. The danger appears to be moderate, and current smokers are 1.5 times more at risk. Sexuality, study design, the number of cases, or the time period of publication did not indicate any significant variations in the risk of liver cancer caused by cigarette smoking (14).

In our study Total protein had significant positive correlation with Albumin, Globulin and ALT and Total Protein had negative correlation with A/G ratio. Globulin had strong positive association with Bilirubin Total and Direct with p value while Globulin had negatively associated with A/G ratio. A/G ratio was negatively correlated with Bilirubin Total and Direct. The association between smoking and reduced serum bilirubin has been examined in a number of earlier research. An earlier investigation discovered a substantial negative correlation between smoking status and bilirubin in both those with and without coronary

artery disease. Male active smokers had significantly lower serum bilirubin amounts than never smokers, according to another study (23).

Although several partial explanations have been proposed, the exact mechanisms underlying the association between cigarette smoke exposure and decreased serum bilirubin levels are not yet fully understood. Bilirubin, a bile pigment, is increasingly recognized for its physiological role as a potent endogenous antioxidant. In vitro studies have demonstrated that bilirubin can effectively prevent the oxidation of low-density lipoprotein (LDL) as well as vitamins C and E, thereby protecting against oxidative stress (24,25). It has also been suggested that the bilirubin–biliverdin redox cycle plays a major cytoprotective role in cells by scavenging reactive oxygen species (ROS) such as free radicals (26). Cigarette smoke is a rich source of ROS, which can overwhelm the antioxidant defense system and potentially degrade circulating bilirubin. This oxidative burden may explain the significantly lower serum bilirubin levels observed in smokers compared to non-smokers (27).

Our study further implies that bilirubin total has been strongly correlated with direct and indirect bilirubin. Bilirubin direct had strong positive association with indirect bilirubin. Indirect bilirubin has been positively associated with liver enzymes, particularly aspartate aminotransferase (AST) and alanine aminotransferase (ALT), both of which are key indicators of hepatic function. Several studies have reported a statistically significant correlation between elevated levels of indirect or total bilirubin and increased AST and ALT levels. This association may reflect hepatocellular injury or impaired bilirubin metabolism (28). In particular, a strong positive relationship between indirect bilirubin and AST has been observed in both hepatitis and metabolic syndrome cohorts, suggesting a shared pathophysiological pathway involving oxidative stress and liver dysfunction (29, 30). An earlier study undertaken at our laboratory suggested that small dense LDL particles evaluated through the surrogate TAG/HDL ratio and thyroid hormone with divalent cations could be used as objective markers for insulin resistance with thyroid comorbidity in overweight and obese type 2 diabetics (31,32).

These findings are consistent with previous reports that tobacco use contributes to dyslipidemia, thereby increasing the risk of cardiovascular disease. Interestingly, in tobacco chewers, age showed a negative relationship with total cholesterol and LDL, suggesting age- or duration-dependent metabolic adaptation. The interaction between tobacco exposure and thyroid hormones suggests that chronic tobacco use may influence thyroid gland activity, possibly predisposing individuals to future thyroid abnormalities.

CONCLUSION:

Tobacco consumption is significantly associated with alterations in liver and thyroid function. The observed biochemical correlations suggest that tobacco use may predispose individuals to hepatic and thyroid disorders. These findings warrant further longitudinal studies to explore causality and inform clinical screening protocols for tobacco users.Understanding these biochemical changes is critical for early diagnosis, risk assessment, and prevention of long-term complications in habitual tobacco users. These findings highlight the significance of regular biochemical and hormonal assessments in tobacco users to detect early indicators of hepatic stress and thyroid dysfunction, potentially exposing them to further metabolic and endocrine diseases.

LIMITATIONS:

1. Cross-sectional design study captures correlations at a single point in time, limiting the ability to infer causality between tobacco use and changes in liver or thyroid function.

2. The study population may not be large or diverse enough to generalize findings across different age groups, genders, or regions.

3. Reliance on self-reported smoking or chewing habits can lead to underreporting or inaccuracies in exposure assessment and accuracy.

4. Only basic thyroid hormones and liver enzymes were assessed.

FUTURE SCOPE:

Future research should focus on long-term follow-up studies to assess the sustained impact of tobacco use on liver and thyroid function. Comparing tobacco users with non-users, and monitoring changes following tobacco cessation, would help establish stronger causal relationships. Additionally, evaluating biochemical variations based on the duration and intensity of tobacco exposure may offer insights into risk thresholds. Future studies should also incorporate oxidative stress markers, inflammatory cytokines, and autoantibodies to better understand the mechanistic pathways through which tobacco contributes to organ dysfunction. Lastly, it is important to investigate whether certain demographic or clinical subgroups are more susceptible to tobacco-induced alterations in liver and thyroid profiles, which could guide targeted interventions.

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CONFLICT OF INTEREST:

The authors declare there is no conflict of interest.

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