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ARTICLE

Differences in Brain Volume and Thickness between Shammah (Smokeless Tobacco) Users and Non-Users: A Cross-Sectional Study

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Abstract

Aims/Background Previous research has shown that smoking tobacco is associated with changes or differences in brain volume and cortical thickness, resulting in a smaller brain volume and decreased cortical thickness in smokers compared with non-smokers. However, the effects of smokeless tobacco on brain volume and cortical thickness remain unclear. This study aimed to investigate whether the use of shammah, a nicotine-containing smokeless tobacco popular in Middle Eastern countries, is associated with differences in brain volume and thickness compared with non-users and to assess the influence of shammah quantity and type on these effects.

Methods Male shammah users (aged 20 to 47 years, n = 30) and non-users (n = 39) underwent 1.5T magnetic resonance imaging (MRI) scans, and cortical brain volumes and thicknesses were measured using FreeSurfer.

Results Significant differences were found in the volume of the right pallidum ($p = 0.02$), total pallidum ($p = 0.02$), total ventricle ($p = 0.02$), middle posterior corpus callosum ($p = 0.04$), and brainstem ($p = 0.02$) between shammah users and non-users. Furthermore, yellow shammah users exhibited smaller volumes in the right lateral ventricle ($p = 0.02$), total lateral ventricle ($p = 0.03$), and right putamen ($p = 0.02$) compared with users of other types of shammah. Regarding cortical thickness, significant differences were observed in the right medial orbito-frontal thickness ($p = 0.03$), left rostral middle frontal thickness ($p = 0.03$), and right rostral anterior cingulate thickness ($p = 0.04$).

Conclusion These findings shed light on the potential neurobiological effects of shammah use, particularly the yellow shammah, and highlighting the need for further research to fully understand its implications for brain structure and function.

Key words: shammah; smokeless tobacco; brain volume; brain cortical thickness; MRI

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Introduction

Tobacco, a highly addictive substance, is widely recognized for its adverse health effects. In developed countries, approximately 22% of adults are frequently users of tobacco products, with a higher prevalence among men (Hitchman and Fong, 2011). Nicotine, a chemical substance present in all tobacco types, is the primary addictive component responsible for tobacco dependence (Haustein and Groneberg, 2010). Smokeless tobacco (ST), which includes chewing tobacco, powdered tobacco mixed with other products, and snuff, is another common form of tobacco use. ST is placed in the mouth near the cheek or lip and either sucked (dipped) or chewed, or inhaled nasally (Alsanosy, 2014; Tate, 2004; Yaldiz et al, 2018). The use of ST has increased globally (Chugh et al, 2023), with more than 300 million adults reported to be ST users, primarily concentrated in South-East Asia (89%) (Bakdash, 2017). ST is particularly prevalent in the Arabian Peninsula, especially in Saudi Arabia and Yemen (Brima, 2016). In Saudi Arabia, especially in the provinces of Jazan, Najran, and Jeddah, a popular ST product called shammah or Yemeni snuff is commonly used. Shammah consists of cut, dried, and milled tobacco leaves mixed with other substances such as black pepper, lime, and oil (Alsanosy, 2014). It is held between the cheek and the gum, and nicotine is absorbed through the oral tissues, with users spitting out insoluble residue. Shammah is classified into four main types based on their constituents, each named after its color: white shammah (also known as shammah beda), red shammah (colored with henna), yellow shammah (pure tobacco powder without additives), and brown shammah (a mixture of yellow and white shammah). The majority of shammah users in the Arabian Peninsula prefer yellow shammah, while new users typically start with white shamma (Brima, 2016). The amount of shammah used per session is usually less than 1.0 gram.

Previous studies of tobacco smokers (Durazzo et al, 2017; Gallinat et al, 2006; Pan et al, 2013) have shown that middle-aged adults have smaller total grey matter volume (GMV) and regional GMV, particularly in the frontal and temporal lobes, amygdala, cingulate, insula, compared with non-smokers, suggesting the vulnerability of these regions (Elbejjani et al, 2019). Other studies (Brody et al, 2004; Gallinat et al, 2006; Karama et al, 2015; Kühn et al, 2010; Liao et al, 2012; Peng et al, 2018; Yu et al, 2013) have reported decreased thickness of the cerebral cortex, smaller grey matter (GM) and white matter (WM) volumes, impaired global cognitive function, and abnormal task-related brain activation in both light and heavy smokers compared with non-smokers. Smokers exhibited significant brain atrophy in GM areas such as the precuneus, inferior frontal gyrus, orbitofrontal cortex, superior temporal gyrus, thalamus, basal ganglia, cingulate cortex, temporal lobe, anterior lobe of the cerebellum, insula, and total hippocampal volume. Collectively, these findings suggest that tobacco smoking affects brain volume and thickness.

While the effects of smoking tobacco, particularly cigarette smoking, on brain structure have been extensively studied, the effects of smokeless tobacco, shammah, on brain morphology or thickness remain unexplored. Understanding the effects of shammah use on the brain is important, because it can provide insights into the potential neurobiological consequences of this habit. The aim of this study is to investigate whether shammah use leads to similar patterns of brain volume and cortical differences compared to controls, as observed in tobacco smokers. Based on previous neuroimaging research demonstrating the effects of tobacco smoking on brain volume, we explored whether shammah users exhibit smaller regional brain volumes and thinner cortices compared with healthy controls.

Methods

Participants

A total of 80 participants, consisting of 40 male shammah users and 40 age-matched male non-users, were initially recruited using snowball sampling (see Fig. 1). Recruitment began by identifying shammah users who met the inclusion criteria, followed by the selection of non-user participants matched in age to each shammah user (within ±2 years), resulting in 40 shammah users and 40 age-matched non-users. Of these, 69 participants (30 shammah users and 39 non-users) were ultimately included in the final analysis, after excluding 11 participants due to magnetic resonance imaging (MRI)-related issues (6 with significant motion artifacts, 3 with unusual lesions that impeded proper FreeSurfer processing, and 2 with incomplete brain coverage in their T1 scans). The effectiveness of the age-matching procedure was verified by the comparable age distributions between the two groups (shammah users: mean age = 28.8 years (±5.98); non-users: mean age = 28.3 years (±6.22); t = –0.368, p = 0.71).

The study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the institutional review board (IRB) of Najran Health Affairs, Najran Hospital, Saudi Arabia (IRB approval number: 2021-39 E). The IRB is registered with King Abdulaziz City for Science and Technology (KACST, https://kacst.gov.sa/) under registration number H-l1-N-081. Specific inclusion and exclusion criteria were established prior to recruitment. Participants were required to be adults aged between 20 and 60 years and residents of the Najran region. Shammah users were included if they reported regular use with a minimum frequency of five sessions per day for at least two years, with no cessation periods exceeding three months. Non-users were required to have no history of shammah or tobacco use. Participants were excluded if they had any history of neurological or general medical disorders, current or past tobacco smoking, participation in pharmacological or behavioral cessation programs, or the use of other forms of tobacco products. Additional exclusion criteria included notable structural brain abnormalities on routine MRI or poor-quality MRI data (excessive motion artifacts, incomplete brain coverage, or processing errors).

Face-to-face interviews were conducted by two trained research assistants with backgrounds in psychology and neurology, who were blinded to the participants' shammah use status. These interviewers followed a standardized interview protocol with specific guidelines and scripts to ensure consistency and reliability across participants and to minimize interviewer bias. Educational level was coded numerically for analysis (0 = no qualification, 1 = diploma degree, 2 = bachelor's degree, 3 = master's degree, and 4 = PhD degree). For shammah users, detailed information was collected on shammah count (or frequency) [i.e., the typical number of shammah sessions per day] and shammah type [yellow shammah or other shammah].

All participants provided written informed consent before participation. Routine head MRI scans were performed prior to data collection to confirm the absence of significant structural brain abnormalities, and that none of the included shammah users were enrolled in pharmacological or behavioral smoking cessation programs or were using any other form of tobacco during the study period.

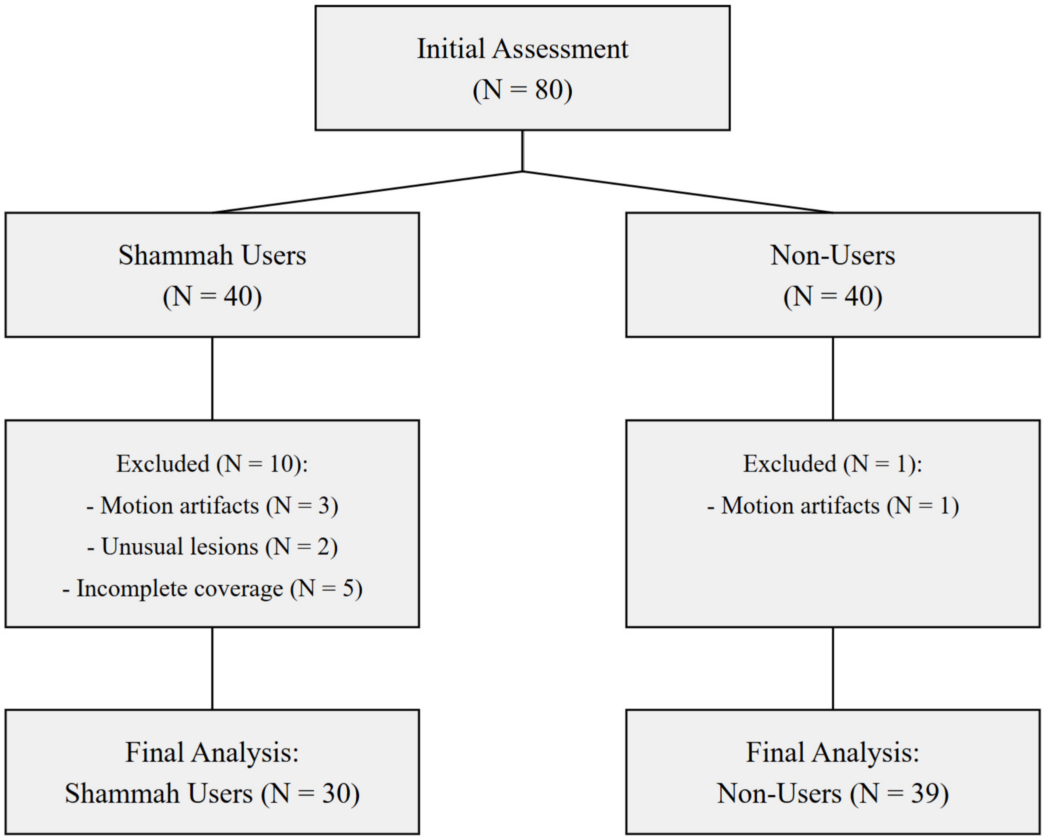

Fig. 1. Participants selection flowchart.

MRI Acquisition

MRI data were collected using a 1.5T Siemens MRI scanner at Najran General Hospital, equipped with a standard 32-channel radio frequency (RF) receive head coil. During the scanning process, participants, both shammah users and non-users, were instructed to stay relaxed and minimize movement. T1-weighted 3D magnetization-prepared rapid gradient echo (MPRAGE) images were captured in the sagittal plane. The imaging session lasted approximately 4 minutes and 59 seconds, with the following settings: 1 × 1 × 1 mm spatial resolution, time repetition (TR) of 2200 ms, inversion time (TI) of 900 ms, time echo (TE) of 2.88 ms, an 8° flip angle, and a field of view (FOV) of 208 × 250 × 250 mm. The integrated parallel acquisition technique (iPAT) factor was set to 2, and the superior-inferior FOV was 250 mm.

Image Processing

Brain volumetric processing, parcellation, and segmentation were conducted using FreeSurfer version 6.0 (Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Boston, MA, USA) (http://surfer.nmr.mgh.harvard.edu) on a Dell computer running Ubuntu 18.0. FreeSurfer was chosen for this analysis due to its well-documented reliability and consistency in both cortical and subcortical segmentations, particularly in longitudinal studies. It has established longitudinal reproducibility in cortical and subcortical segmentations (Glatard et al, 2015; Ochs et al, 2015), as well as robust reliability in morphometric measurements across different scanner manufacturers and field strengths (Reuter et al, 2012). Furthermore, FreeSurfer demonstrates superior reproducibility in subcortical segmentations compared with alternative automated methods (Velasco-Annis et al, 2018). Initially, all MRI data were converted to the Neuroimaging Informatics Technology Initiative (NIFTI) format, followed by manual inspection of each subjects T1 scan to identify and address any motion artifacts or quality issues. FreeSurfer employs probabilistic information from a manually labelled training set to assign neuroanatomical labels to voxels in MRI volumes with cortical and subcortical regions of interest. The software performs various data processing steps, including averaging volumetric T1-weighted images, skull stripping to remove non-brain tissues, motion correction, transformation to Talairach image space, intensity normalization, removal of non-brain tissues using a hybrid watershed, and segmentation of subcortical volumetric structures, white matter (WM), and deep grey matter (GM) (Fischl et al, 2002) (see Fig. 2). To account for potential group differences between the left and right hemispheres of bilateral regions, cortical and subcortical volumetric measures were averaged for each side (Potvin et al, 2017). A total of 27 regional cortical and subcortical volumes were included in the final analysis. These brain volumes were normalized by the total brain volume (TBV) to adjust for variations in head size between individuals (Barnes et al, 2010). In addition, FreeSurfer calculated other brain volumes such as TBV and intracranial volume (ICV) using the Talairach transformation matrix based on normalization and the Montreal Neurological Institute (MNI) atlas (Barnes et al, 2010). While FreeSurfer does not directly measure white matter volume (WMV), it can be calculated by summing distinct regions such as cerebral WM, cerebellar WM, brainstem, and corpus callosum (Lin et al, 2015). The segmentation and processing steps were performed using default settings, and the resulting volumes were extracted using specific commands. Cortical thickness values were also obtained from FreeSurfer's output, which computes the mean distance between the vertices of a triangulated surface representing the GM/WM boundary and the GM/cerebrospinal fluid boundary. Visual checks were conducted to ensure the accuracy of the processing, parcellations, and segmentations.

Statistical Analyses

Data analysis was performed using SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA), with a significance level of 0.05 for all two-tailed hypothesis tests. ANCOVA analyses were used to examine the differences in brain

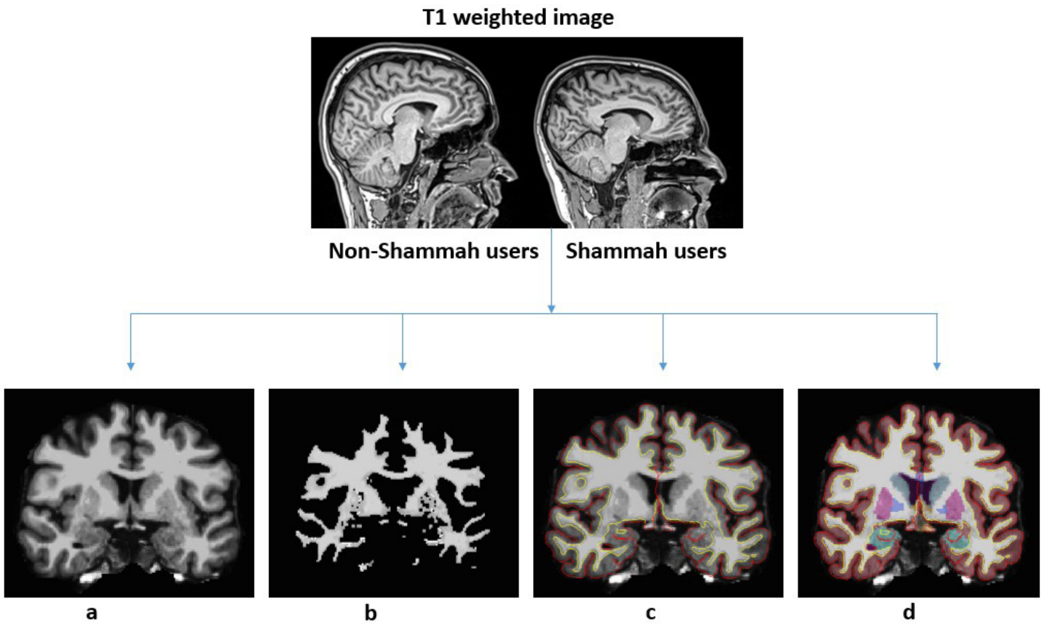

Fig. 2. Processing pipeline for structural MRI data. (a) Skull stripping. (b) Bias field correction and GM/WM segmentation. (c) Reconstruction of cortical surface models (GM boundary surface and pial surface). (d) Labelling of regions on the cortical surface, as well as subcortical brain structures. MRI, magnetic resonance imaging; GM, grey matter; WM, white matter.

volume or thickness between the shammah user and non-user groups, followed by post hoc tests. The assumptions of normality and homogeneity of variance were verified prior to performing the ANCOVA analyses. The normality of residuals was assessed using the Shapiro Wilk test, and the homogeneity of variance was evaluated using Levenes test. Variables that deviated from normality ($p < 0.05$) were identified as non-normally distributed. To address this non-normality, a Box-Cox transformation, which optimizes the normality of the data through a power transformation, was applied to the non-normally distributed columns. Furthermore, outlier detection was performed by using the z-score method. Rows with z-scores exceeding a predetermined threshold of 3 were identified as outliers and subsequently removed from the dataset. Separate ANCOVA analyses were conducted to investigate the association between shammah use, type, and count and changes in brain volumes and thicknesses. Age and TBV were included as covariates in the ANCOVA model to account for their associations with brain volume and thickness in previous studies (Cox et al, 2016; Majrashi et al, 2020; Majrashi et al, 2022; Peters, 2006). To examine the potential influence of shammah count (or frequency) on the observed differences between types of shammah users, we included shammah count as a covariate in our ANCOVA analyses. Statistical significance for the ANCOVA main effects was set at $p < 0.05$. Following the ANCOVA analysis, post-hoc t-tests were conducted. Covariates were compared between shammah users and non-users using independent samples t-tests. The characteristics of shammah users and non-users were analyzed using independent sample t-tests for continuous variables, such as age, body mass index (BMI), weight, and height. Categorical variables, such as educational level and living area, were analyzed using either Fisher's exact test or the chi-square test, depending on the characteristics of the data. Fisher's exact test was used to analyze educational level due to its suitability for small sample sizes and categorical distributions, while the chi-square test was employed to analyze the 'living area' variable. In secondary statistical analyses, shammah users were further categorized based on the number of sessions per day (less than 10 vs. more than 10) and shammah type (yellow shammah vs. others). Given the exploratory nature of this initial investigation into potential structural brain differences between shammah users and non-users, uncorrected *p*-values were reported conservatively.

Results

Participant Characteristics

Eighty male participants were enrolled in the study, with an equal distribution of 50% shammah users and 50% age-matched non-users. Of the 80 participants, 69 individuals (30 shammah users and 39 age-matched non-users) ranging in age from 20 to 47 years (mean = 28.5, SD = 6.1) were included in this analysis. Eleven participants were excluded due to issues with their T1 MRI images during the automated processing conducted by FreeSurfer. Specifically, 6 participants had significant motion artifacts or other image quality issues that prevented successful segmentation and parcellation by FreeSurfer. An additional 3 participants had unusual lesions that the FreeSurfer algorithms were unable to properly process. Finally, 2 participants had incomplete coverage of the brain volume in their T1 scans, likely due to positioning issues during the MRI acquisition. No significant differences were observed between shammah users and non-users in terms of demographic variables such as age, height, and education (see Table 1) or TBV or ICV (Table 2). However, there were significant differences in weight ($t = 2.19$, $p = 0.03$) and BMI ($t = 2.06$, $p = 0.04$) between shammah users and non-users, as indicated in Table 1. Detailed demographic characteristics and brain volumes and thicknesses for each group can be found in Tables 1,2,3.

Comparison of GM, WM, and Regional Brain Volumes between Shammah Users and Non-Users

The results revealed significant associations between shammah use and differences in several brain regions, including the right pallidum (F[4] = 5.31, $p = 0.02$), corpus callosum (CC) middle posterior (F[4] = 4.37, $p = 0.04$), total pallidum (F[4] = 5.30, $p = 0.02$), whole brainstem (F[4] = 5.64, $p = 0.02$), and total ventricles (F[4] = 5.20, $p = 0.02$). The notation "F[4]" refers to the F-statistic obtained from the ANCOVA analysis, where the number "4" indicates the degrees of freedom for the numerator (i.e., the number of groups or factors being compared). Trends were observed for the left cerebellum (F[4] = 3.27, $p = 0.07$), left pallidum (F[4] = 3.11, $p = 0.08$), right cerebellum (F[4] = 3.69, $p = 0.06$), and total cerebellum (F[4] = 3.61, $p = 0.08$). Follow-up *t*-tests revealed that shammah users had significantly larger volumes than non-users in the right pallidum (ES = 0.37), CC middle posterior (ES = 0.38), total pallidum (ES = 0.34), brainstem (ES = 0.22), and total ventricles (ES = 0.46). No other significant differences in brain volumes were observed between shammah users and non-users.

Table 1. Characteristics of shammah users and non-users.

Variable	All participants (N = 69)	Shammah users (N = 30)	Non-users (N = 39)	Statistical test	p values
Number of participants, N (%)	69 (100%)	30 (43.5%)	39 (56.5%)		
Age (years), mean (SD)	28.5 (6.10)	28.8 (5.98)	28.3 (6.22)	$t = -0.368$	0.71
BMI, mean (SD)	20.7 (3.76)	19.7 (3.36)	21.5 (3.91)	$t = 2.06$	0.04
Weight (kg), mean (SD)	71.3 (13.1)	67.5 (11.1)	74.2 (13.9)	$t = 2.19$	0.03
Height (cm), mean (SD)	171.8 (4.80)	171.4 (5.23)	172.1 (4.62)	$t = 0.576$	0.56
Educational level, N (%)	1.29 (0.98)	1.16 (0.920)	1.39 (1.02)		0.41
0 (N)	18	10	8		
1 (N)	21	7	14		
2 (N)	25	12	12		
3 (N)	5	1	4		
4 (N)	1	0	1		
Living area, N (%)				$\chi^2 = 1.14$	0.28
Rural	25 (36.2%)	9 (30.0%)	17 (43.5%)		
Urban	44 (63.8%)	21 (70.0%)	22 (56.4%)		
Shammah types, N (%)					
Yellow shammah	-	18 (60.0%)	-		
Brown shammah	-	11 (36.7%)	-		
Red shammah	-	1 (3.3%)	-		
Shammah count (frequency), N					
Shammah count (frequency) <10 times (Yellow/Brown/Red)	-	7/6/1	-		
Shammah count (frequency) >10 times (Yellow/Brown/Red)	-	11/5/0	-		

Abbreviations: BMI, body mass index; N, number of participants. Educational level was coded as follows: 0 = no qualification, 1 = diploma degree, 2 = bachelor degree, 3 = master degree, and 4 = PhD degree. Values represent the mean (SD).

Table 2. Measurements of cortical-based brain volumes for both shammah users and non-users.

Brain volumes (mm^3)	Shammah users (N = 30)		Non-users (N = 39)		*p* value
	Mean	SD	Mean	SD	
Left lateral ventricle	7255.0	3379.6	8515.5	3300.6	0.15
Left cerebellum	73,417.2	5513.4	73,025.6	5757.4	0.07
Left thalamus	7749.0	803.3	7815.8	692.0	0.27
Left caudate	3485.4	408.0	3540.3	384.5	0.70
Left putamen	4996.9	520.8	5139.6	534.8	0.09
Left pallidum	2043.5	224.7	2022.9	181.9	0.08
Left hippocampus	4020.4	417.2	4155.1	354.6	0.34
Left amygdala	1588.8	209.0	1664.3	197.6	0.10
Left accumbens area	566.2	79.6	579.3	91.2	0.65
Left ventral-DC	4058.1	270.7	4100.4	342.5	0.45
Left choroid plexus	499.3	164.3	530.1	150.9	0.45
Right lateral ventricle	6253.7	3034.5	7901.1	3945.7	0.23
Right cerebellum	73,282.1	5860.5	73,246.1	6297.9	0.06
Right thalamus	7476.2	815.3	7555.3	620.3	0.15
Right caudate	3622.5	486.5	3635.5	416.0	0.09
Right putamen	5113.8	548.1	5205.8	578.4	0.27
Right pallidum*	1992.3	160.3	1947.5	167.6	0.02
Right hippocampus	4206.4	348.8	4296.2	364.4	0.33
Right amygdala	1722.4	223.8	1760.1	199.7	0.34
Right accumbens area	578.7	80.2	619.0	87.1	0.10
Right ventral-DC	4013.7	303.1	4099.3	315.6	0.65
Right choroid plexus	583.0	156.7	608.0	170.5	0.33
Whole brainstem*	21,805.8	1598.5	21,562.5	1730.6	0.02
CSF	949.7	169.4	1025.8	200.7	0.59
WM-hypointensities	970.7	267.4	918.6	225.3	0.18
CC posterior	1009.9	172.7	1021.4	147.8	0.08
CC middle posterior*	581.2	119.2	542.7	84.8	0.04
CC central	551.4	113.6	516.1	92.9	0.16
CC middle anterior	556.6	115.1	534.8	99.8	0.09
CC anterior	895.0	183.0	900.9	157.2	0.50
Lateral ventricles	13,508.7	6197.3	16,416.6	7002.4	0.10
Total lateral ventricle	13,508.7	6197.3	16,416.6	7002.4	0.40
Total thalamus	15,225.2	1501.5	15,371.1	1250.8	0.22
Total caudate	7107.9	874.0	7175.8	787.2	0.28
Total putamen	10,110.8	1000.5	10,345.3	1083.7	0.66
Total pallidum*	4035.8	344.7	3970.4	333.5	0.02
Total hippocampus	8226.8	737.5	8451.3	679.6	0.33
Total amygdala	3311.2	399.1	3424.4	373.2	0.34
Total accumbens area	1144.9	136.3	1198.3	167.3	0.10
Total ventral-DC	8071.8	554.9	8199.8	645.1	0.65
Total choroid plexus	1082.4	297.3	1138.2	299.3	0.22

Table 2. Continued.

Brain volumes (mm^3)	Shammah users (N = 30)		Non-users (N = 39)		*p* value
	Mean	SD	Mean	SD	
Total cerebellum	146,699.3	11,216.8	146,271.7	11,940.2	0.08
Total ventricles*	2666.3	578.4	2952.0	594.1	0.02
WMV (cm^3)	483.7	48.2	494.3	41.1	0.34
GMV (cm^3)	671.0	46.1	687.7	53.5	0.10
TBV (cm^3)	1154.7	84.4	1182.1	86.6	0.65

Abbreviations: CSF, cerebrospinal fluid; DC, diencephalon; CC, corpus callosum; WMV, white matter volume (cm^3); GMV, grey matter volume (cm^3); TBV, total brain volume (cm^3). Total (left side + right side). * = the brain regions where there are differences in volume between shammah users and non-users.

Regarding shammah type, a significant effect was observed in the right lateral ventricle (F[4] = 5.77, $p = 0.02$), right putamen (F[4] = 5.89, $p = 0.02$), and total lateral ventricle (F[4] = 4.98, $p = 0.03$). Follow-up *t*-tests indicated that yellow shammah users had significantly smaller volumes in these brain regions compared to users of other shammah types. After adjusting for shammah count, the differences in brain volume between yellow and other shammah users remained significant, $p < 0.05$. The results indicate that while shammah count is an important factor, the type of shammah used has an independent effect on brain structure. No other significant differences were observed. In terms of shammah frequency, ANCOVA analyses revealed no significant effects on brain volume in any region.

Comparison of Cortical Thickness between Shammah Users and Non-Users

The results revealed significant associations between shammah use and specific brain region thicknesses, including right medial orbito-frontal thickness (F[3] = 4.89, $p = 0.03$), left rostral middle frontal thickness (F[3] = 4.71, $p = 0.03$), and right rostral anterior cingulate thickness (F[3] = 4.15, $p = 0.04$). Follow-up t-tests demonstrated that shammah users had significantly smaller thicknesses than non-users in the right medial orbito-frontal (ES = 0.45), right rostral anterior cingulate (ES = 0.39), and left rostral middle frontal (ES = 0.35) regions. No other significant differences in brain thickness were observed between shammah users and non-users.

When comparing different types of shammah, no significant effects on cortical brain thickness were observed ($p > 0.05$). After adjusting for shammah count, the differences in brain volume and cortical thickness between users of yellow shammah and other types of shammah remained insignificant. Similarly, ANCOVA analyses showed no significant association between shammah frequency and brain region thickness ($p > 0.05$).

Discussion

To our knowledge, the current study is the first to demonstrate differences in neuroanatomical structure between shammah users and non-users. We found that

Table 3. Measurements of cortical-based brain thickness for shammah users and non-users.

Brain thickness (mm^2)	Non-shammah users (N = 39)		Shammah users (N = 30)		*p* value	Brain thickness (mm^2)	Non-shammah users (N = 39)		Shammah users (N = 30)		*p* value
	Mean	SD	Mean	SD			Mean	SD	Mean	SD	
rh_bankssts_thickness	2.47	0.19	2.48	0.16	0.23	lh_bankssts_thickness	2.41	0.11	2.41	0.17	0.42
rh_caudalanteriorcingulate_thickness	2.28	0.14	2.27	0.17	0.25	lh_caudalanteriorcingulate_thickness	2.31	0.20	2.38	0.21	0.29
rh_caudalmiddlefrontal_thickness	2.49	0.15	2.45	0.15	0.75	lh_caudalmiddlefrontal_thickness	2.48	0.12	2.43	0.14	0.54
rh_cuneus_thickness	1.81	0.13	1.79	0.14	0.99	lh_cuneus_thickness	1.75	0.12	1.77	0.13	0.70
rh_entorhinal_thickness	3.38	0.25	3.33	0.27	0.60	lh_entorhinal_thickness	3.21	0.20	3.23	0.34	0.31
rh_fusiform_thickness	2.70	0.09	2.69	0.10	0.91	lh_fusiform_thickness	2.69	0.09	2.66	0.10	0.61
rh_inferiorparietal_thickness	2.36	0.11	2.37	0.10	0.73	lh_inferiorparietal_thickness	2.41	0.11	2.40	0.15	0.70
rh_inferiortemporal_thickness	2.87	0.12	2.85	0.12	0.07	lh_inferiortemporal_thickness	2.86	0.10	2.86	0.11	0.51
rh_isthmuscingulate_thickness	2.21	0.14	2.18	0.16	0.60	lh_isthmuscingulate_thickness	2.20	0.12	2.21	0.14	0.19
rh_lateraloccipital_thickness	2.16	0.10	2.14	0.10	0.96	lh_lateraloccipital_thickness	2.06	0.08	2.08	0.11	0.93
rh_lateralorbitofrontal_thickness	2.59	0.13	2.56	0.11	0.18	lh_lateralorbitofrontal_thickness	2.61	0.11	2.58	0.12	0.80
rh_lingual_thickness	1.97	0.09	1.97	0.09	0.15	lh_lingual_thickness	1.98	0.09	1.96	0.10	0.42
rh_medialorbitofrontal_thickness*	2.43	0.13	1.98	0.11	0.03	lh_medialorbitofrontal_thickness	2.46	0.12	2.45	0.13	0.29
rh_middletemporal_thickness	2.88	0.09	2.90	0.13	0.14	lh_middletemporal_thickness	2.91	0.12	2.91	0.15	0.54
rh_parahippocampal_thickness	2.61	0.19	2.65	0.21	0.94	lh_parahippocampal_thickness	2.69	0.23	2.70	0.26	0.70
rh_paracentral_thickness	2.29	0.13	2.32	0.14	0.49	lh_paracentral_thickness	2.26	0.16	2.30	0.14	0.31
rh_parsopercularis_thickness	2.58	0.23	2.60	0.15	0.08	lh_parsopercularis_thickness	2.56	0.16	2.54	0.12	0.61
rh_parsorbitalis_thickness	2.76	0.19	2.71	0.13	0.07	lh_parsorbitalis_thickness	2.72	0.20	2.67	0.21	0.70
rh_parstriangularis_thickness	2.42	0.15	2.46	0.14	0.16	lh_parstriangularis_thickness	2.37	0.14	2.36	0.13	0.51
rh_pericalcarine_thickness	1.50	0.12	1.49	0.10	0.08	lh_pericalcarine_thickness	1.49	0.10	1.50	0.06	0.19
rh_postcentral_thickness	1.97	0.15	1.98	0.12	0.80	lh_postcentral_thickness	1.96	0.11	1.96	0.11	0.93
rh_posteriorcingulate_thickness	2.27	0.10	2.27	0.11	0.19	lh_posteriorcingulate_thickness	2.30	0.11	2.29	0.13	0.80
rh_precentral_thickness	2.48	0.17	2.43	0.19	0.14	lh_precentral_thickness	2.48	0.17	2.44	0.15	0.42
rh_precuneus_thickness	2.28	0.10	2.27	0.12	0.94	lh_precuneus_thickness	2.27	0.11	2.28	0.12	0.29
rh_rostralanteriorcingulate_thickness*	2.65	0.15	1.91	0.16	0.04	lh_rostralanteriorcingulate_thickness	2.73	0.18	2.72	0.19	0.54

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Table 3. Continued.

Brain thickness (mm^2)	Non-shammah users (N = 39)		Shammah users (N = 30)		*p* value	Brain thickness (mm^2)	Non-shammah users (N = 39)		Shammah users (N = 30)		*p* value
	Mean	SD	Mean	SD			Mean	SD	Mean	SD	
rh_rostralmiddlefrontal_thickness	2.31	0.11	2.32	0.10	0.70	lh_rostralmiddlefrontal_thickness*	2.34	0.12	1.90	0.11	0.03
rh_superiorfrontal_thickness	2.70	0.11	2.69	0.11	0.31	lh_superiorfrontal_thickness	2.70	0.13	2.68	0.12	0.38
rh_superiorparietal_thickness	2.07	0.12	2.10	0.11	0.61	lh_superiorparietal_thickness	2.09	0.12	2.09	0.12	0.07
rh_superiortemporal_thickness	2.77	0.14	2.76	0.13	0.70	lh_superiortemporal_thickness	2.78	0.15	2.76	0.10	0.52
rh_supramarginal_thickness	2.43	0.14	2.43	0.11	0.51	lh_supramarginal_thickness	2.47	0.13	2.46	0.13	0.16
rh_frontalpole_thickness	2.68	0.29	2.65	0.31	0.08	lh_frontalpole_thickness	2.70	0.27	2.73	0.24	0.38
rh_temporalpole_thickness	3.59	0.41	3.74	0.25	0.07	lh_temporalpole_thickness	3.51	0.39	3.57	0.26	0.08
rh_transversetemporal_thickness	2.26	0.20	2.23	0.24	0.16	lh_transversetemporal_thickness	2.20	0.21	2.19	0.21	0.07
rh_insula_thickness	3.01	0.13	3.00	0.12	0.08	lh_insula_thickness	2.95	0.13	2.94	0.11	0.16
rh_MeanThickness_thickness	2.42	0.08	2.42	0.07	0.80	lh_MeanThickness_thickness	2.43	0.08	2.41	0.08	0.08

Abbreviations: rh, right; lh, left. * = the brain regions where there are differences in thickness between shammah users and non-users.

shammah users, when compared with non-users, had larger brain volumes in the right pallidum, CC middle posterior, total pallidum, whole brainstem, and total ventricles, but smaller brain thickness in the right medial orbital frontal, right rostral anterior cingulate, and left rostral middle frontal regions, after correcting for covariates including age and TBV (for only volume). We also found that yellow shammah users exhibited smaller volume in the right lateral ventricle, right putamen, and total lateral ventricle than those who use red and brown shammah. These findings suggest that shammah (ST), especially the yellow shammah, affects regional cortical volumes and thicknesses.

The observed differences in brain volumes between adult shammah users and non-users are consistent with previous studies of smoking-related tobacco use, which found that young and middle-aged adult smokers had larger volumes than non-smokers in GM cortical regions such as the bilateral putamen and para-hippocampus (Franklin et al, 2014; Wetherill et al, 2015). Moreover, the differences in cortical brain thicknesses are consistent with previous studies (Durazzo et al, 2013; Karama et al, 2015), which have shown that cigarette smoking is associated with cortical thinning in several regions of the brain such as prefrontal areas, frontal cortex, orbito-frontal cortex, and global cortex after controlling for covariates, and that smokers exhibit a thinner cortex compared to non-smokers. In addition, there was a trend toward differences in the volume of other brain regions including the cerebellum and pallidum. While significant differences in brain volumes were observed in the brainstem, pallidum, and CC, no differences were found in other brain volumes such as total GM, WM, CSF, amygdala, and hippocampus between shammah users and non-users. These findings are consistent with previous research (Block et al, 2000; Gallinat et al, 2006; Shen et al, 2017; Tzilos et al, 2005) on smokers, which also reported no significant differences in these brain volumes compared with non-smokers.

In addition, our study found significant differences in brain volume in regions critical for mood and addiction, namely the brainstem, pallidum, and corpus callosum. The brainstem contains reticular nuclei, which include monoamine-producing nuclei that distribute throughout the brain (Naidich et al, 2009). The brainstem plays a crucial role in regulating mood, and emotional functions (Song et al, 2014; Venkatraman et al, 2017). The pallidum, an integral component of the basal ganglia, and plays an essential role in motor control and the modulation of movement (Kita, 2007). However, mood disorders, such as depression and bipolar disorder, have also been linked to dysfunctions in the pallidum (Russo and Nestler, 2013). The corpus callosum is a bundle of WM fibres that provides a connection between the cerebral hemispheres, enabling effective communication and the efficient transfer of information between the two sides. Differences in volume are likely to lead to differences in cognitive processing.

Our study showed that yellow shammah users had smaller volume in the putamen and lateral ventricles. Yellow shammah contains pure tobacco leaves without any additional substances, unlike other shammah varieties that contain tobacco along with various additives. The addition of other substances has the effect of diluting the concentration of nicotine in each dose, thereby reducing its neurotoxic effects. Nicotinic acetylcholine receptors in the putamen and dorsal striatum are considered an important part of the mesolimbic dopamine pathway and that striatal abnormalities underlie habitual and compulsive drug seeking and use despite negative consequences (Everitt et al, 2008; Koob and Volkow, 2010). Our finding of smaller putamen volumes in yellow shammah users suggests that the dopamine system may be particularly sensitive to the effects of nicotine.

The current study has several limitations. First, only male, young adult shammah users were recruited to our study as most shammah users in Saudi Arabia are male (Bakdash, 2017). This means that the results may not be generalizable to the wider population. Alternatively, a high proportion of male users may reflect the wider population and so the sampling profile of this study would allow for generalization to the wider population. Second, as a cross-sectional study, we cannot determine the casual relationship between shammah use and volume differences in cortical or subcortical brain regions. Furthermore, as the first study to investigate the potential effects of shammah use, we believe that these uncorrected results suggest associations between shammah use and brain changes that should be further investigated in larger studies.

Conclusion

This study is the first to demonstrate differences in neuroanatomical structure in shammah users compared with non-users, and that yellow shammah users have a significantly greater effect compared with those using other types of shammah. This finding provides further evidence supporting the potential negative effects of non-smoked nicotine containing products on brain structure.

Key Points

- This study investigates the association between shammah, a smokeless tobacco product, and changes of brain volume and cortical thickness in male users compared with non-users.
- Significant differences were found in the volume of specific brain regions, including the right and total pallidum, CC middle posterior, total ventricles, and brainstem, between shammah users and non-users.
- Yellow shammah users exhibited smaller volumes in the right and total lateral ventricle and right putamen compared to users of other shammah types, indicating a potential neurotoxic effect of this variant.
- The findings highlight the potential neurobiological impact of shammah use, emphasizing the need for further research on the long-term effects of smokeless tobacco products on brain health.

Availability of Data and Materials

The data that support the findings of this study are not publicly available due to privacy and confidentiality concerns.

Author Contributions

NAM, ASA, GDW, and TAR contributed to the study conception and design. NAM, MAA, MHAly, MHAlm, EA, ASA, WAA, and YM, performed data collection and analysis. The first draft of the manuscript was written by NAM, NS, BA, AMH, and GDW, and all authors contributed to important editorial changes in the manuscript. NAM and NS contributed to the analysis and interpretation of data. NAM and BA were involved in the acquisition of data, and AMH contributed to the study design and critical revisions of the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Institutional Review Board at Najran Health Affairs, Najran Hospital, Saudi Arabia (Approval Number: [2021-39 E], under the registration number [KACST, KSA: H-l1-N-081]). All participants provided written informed consent to participate in the study. The study was conducted in accordance with the principles outlined in the Decaration of Helsinki.

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Conflict of Interest

The authors declare no conflict of interest.

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