ASSESSMENT OF CEREBRAL PERFUSION IN CHRONIC TOBACCO USERS THROUGH SPECT (SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY) USING TC-99M HMPAO (METASTABLE TECHNETIUM-99 LABELLED HEXA-METHYL PROPYLENE AMINE OXIME)

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ABSTRACT

Objective: To determine an association between chronic tobacco use and changes in cerebral perfusion through semi-quantitative scintigraphic assessment employing metastable Technetium-99 labelled hexa-methyl propylene amine oxime single photon emission computed tomography (Tc-99m HMPAO SPECT).

Design: Case-control study.

Place and duration of study: The study was conducted at the Department of Medical Sciences, Pakistan Institute of Engineering and Applied Sciences (PIEAS), Nilore, Islamabad, from Oct 2001 to May 2002.

Patients and Methods: Regional cerebral perfusion in 48 chronic tobacco users was evaluated, utilising a normal database created by HMPAO brain scans of 20 non-tobacco users. Subjects were classified into chronic tobacco users and non-tobacco users through the use of the smoking index (SI). [Smoking Index = Number of years of tobacco use x Number of cigarettes smoked per day]. SI value of 100 was taken as the cut-off value. Regions of interest (ROIs) were declared hypoperfused or hyperperfused if their percentage perfusion values relative to the average perfusion per pixel of the whole slice under evaluation did not fall within + 2 standard deviation (SD) of the mean regional perfusion in the corresponding ROI, in the normal control group.

Result: Chronic tobacco users showed 164 hypoperfused ROIs (6.57%) and 138 hyperperfused ROIs (5.53%) out of 2496 ROIs assessed, whereas the normal controls showed only 15 hypoperfused (1.44%) and 27 hyperperfused (2.60%) ROIs out of 1040 ROIs assessed (P < 0.001).

Conclusion: Cerebral perfusion in chronic tobacco users was found to be significantly decreased compared to the non-tobacco users.

Keywords: Brain SPECT, Cerebral perfusion, Tobacco, Tc-99m HMPAO, HMPAO SPECT.

INTRODUCTION

There is limited research on the effects of tobacco on cerebral perfusion in individuals who have been tobacco users over prolonged periods. The reason may be difficulties in excluding chronic effects of tobacco use from its acute effects and that cerebral perfusion changes may be seen because of multiple factors such as age, sex, form of tobacco used and its frequency, etc. As nuclear medicine techniques like SPECT employing semi-quantification, sufficiently accurately demonstrate changes in perfusion through the utilization of radiopharmaceuticals, cerebral perfusion SPECT can cost-effectively fill a void in the understanding of long-term effects of tobacco use on cerebral perfusion and has been successfully used for similar purposes in the past.

Tobacco use today, includes consumption of cigarettes, cigars, snuff, chewing tobacco, and loose tobacco for pipe-smoking or hand-rolled cigarettes. In South Asia, a special form of oral tobacco concoction, Naswaar is in common use. Made by crushing tobacco leaves manually or by a mechanical process, Naswaar is put in the mouth for sometime and then discarded. The effect lasts from 1-3 hours. As its sale is not officially regulated and taxed, it is quite cheap and readily available in Pakistan, India and Afghanistan and is in common use.

The most potent tobacco ingredient, Nicotine (1-methyl-2-[3-pyridyl 1] pyrrolidine) is responsible for causing tobacco related effects

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and habituation. It makes up nearly 1 to 2% of tobacco leaves and is a highly toxic alkaloid that causes stimulation of the autonomic ganglia and central nervous system. Nicotine is one of the most widely used psycho-stimulating drugs today[1,2]. Both the positive psychological effects of intake as well as the negative effects of abstinence often explain the urge for nicotine intake[3].

The brain is an organ with almost no energy stock. Neuronal activity depends on the cerebral blood flow (CBF) which provides oxygen and glucose. The CBF is coupled to neuronal activity[4]. This involves an active system of mediators and neurohumoral substances, including potassium, hydrogen, CO2, and adenosine, which are excreted to the extracellular space after oxidative metabolism during increased neuronal activity and result in vasodilator response and hyperaemia[5,6].

Semiquantitative analysis of brain SPECT is done through the percentage ratio between the number of counts in a particular brain region and an identical region, located on the contralateral side, as well as the percentage ratio between the tracer uptake within this region and the tracer uptake in the reference region, e.g., the cerebellum. Normalisation of the average regional counts seen in an image or slice (obtained at a particular level) with the total slice counts, average counts per pixel, or total reconstructed brain counts; or comparison of regional counts with counts from the pons are all among the various methods adopted for rCBF measurement through SPECT. Preferably, any reference region used must have anatomic and functional integrity[7]. The slices to be studied may be transaxial, coronal, sagittal or cut parallel to the orbitomeatal line (OML). The semiquantitative method is particularly useful in defining pathological changes for which the difference in the tracer uptake in both hemispheres does not exceed 10%. This study was done to determine an association between chronic tobacco use and changes in cerebral perfusion (hypoperfusion or hyperperfusion), through semi-quantitative scintigraphic assessment employing Tc-99m HMPAO SPECT

PATIENTS AND METHODS

The research project was conducted at the Department of Medical Sciences, P.I.E.A.S. (Pakistan Institute of Engineering and Applied Sciences) at Nilore, Pakistan. The Ethics Committee for Medical Research at PIEAS approved the study and it was completed over a period of seven months from October 2001 to May 2002.

Patient Selection – Volunteers male chronic tobacco users and non-tobacco users were selected randomly with attempts at eliminating bias emanating from age, social status, state of health and nutrition, as it was difficult to find female chronic tobacco users in the study setting. Diabetes, head injury, epilepsy, transient ischaemic attacks (TIA), Electroconvulsive therapy (ECT), cerebrovascular disease, or any major psychiatric ailment, were excluded.

Informed consent and personal particulars / history were obtained prior to the subject's brain perfusion scan. Detailed history of tobacco use, including type of tobacco used, duration, quantity and frequency of use, mode of consumption and any other addictions were recorded.

Grouping of Data – Smoking Index (SI) was used as a criterion for subject selection for the study, in consonance with similar studies in the past[8-10]. It was calculated through the following relation:

Smoking Index = (Number of cigarettes/ day) x (Years of smoking history)

The patients whose Smoking Index was more than 100, were designated 'chronic tobacco users'. Those who did not smoke, who smoked very occasionally or not enough to be included in this group were placed in the non-tobacco users group (normal controls). The problem posed, by Naswaar users, whose tobacco use could not be adequately judged through the use of Smoking Index (SI), was solved empirically after scrutiny and interviews. A relationship was worked out, which concentrated on the total amount of Naswaar consumed and thus, 01 small sachet of

Naswaar (25 grams) per day was equated to a consumption of 40 cigarettes / day.

Group 1 (Normal controls): The group comprised 20 volunteer, male subjects, normal, healthy, preferably young, 'non-tobacco users'. These included 16 absolute non-tobacco users, 3 very occasional tobacco users and 1 ex-smoker (SI = 40).

Group 2 (Chronic tobacco users): The group comprised 48 'chronic tobacco users', who had been using tobacco in any form over varied lengths of time and had a cumulative SI of 100 or more. These included normal healthy subjects using only one form of tobacco over protracted periods, e.g., chronic smokers; and those using two or more forms of tobacco over protracted periods, e.g., chronic smokers plus Naswaar users.

Protocol for SPECT using Tc-99m HMPAO based on guidelines given in the manual of socity of nuclear medicine[11].

Subject preparation – Pre-arrival: No tobacco use in any form was allowed, at least 3 hours prior to the study and copious fluid intake was encouraged. Subject was asked to void before the study to maximise comfort and limit unnecessary movements.

Pre-injection: Patient was adequately briefed and consistent, calm environment at the time of injection and uptake was ensured.

Injection: There was no stimulatory interaction with the patient, prior or during injection.

Post-injection: No stimulatory interaction with the patient upto 10 minutes post-injection.

Image acquisition: Acquisition was completed within three hours of radiopharmaceutical injection.

Instrumentation – Gamma camera: Single head SPECT camera (Siemens Integrated Orbiter® System) interfaced with Apple Macintosh® Power PC model 8100/110, employing ICON® software version 6.0.3, with parallel hole high-resolution collimator, electronic window width of 15%, centred for Tc-99m at 140 keV, matrix size of 128 x 128 and pixel size of 3.3241 mm. The data was acquired in a circular orbit, in 64 projections, 30 seconds each, through a 360° SPECT range, in step and shoot mode, over 35 to 40 minutes.

Radiochemical purity: Radiochemical purity of the radiopharmaceutical was checked through the use of recommended 3-system chromatographic technique[12,13] and analysis of the Whatman/ITLC paper strips using a gas detector interfaced with a printer.

Image processing: Raw data was processed through ICON software (version 6.0.3), provided with the gamma camera. Brain quantification programme was used for the semi-quantitative analysis of cerebral perfusion.

Treatment of Raw Data and Slice Orientation – Raw data from the acquisitions, with a zoom factor of '1', was reconstructed using Butterworth filter (cut-off = 0.30 cycles / cm; and order = 12), oblique orientation of the images was done in the orbito-meatal line (OML) to obtain 1-pixel thick slices for interpretation of images[14]. Chang's attenuation coefficient value of 0.12 / cm was used, with a lower window level setting of 0% and an upper window level setting of 95%.

Selection of Images and Final Processing – For final interpretation, number of slices per image was increased to three (hence an image was formed by a 3-pixel slice, with a width of 9.97 mm). The default threshold value of 45% was used for the counts in the final images and a Display zoom factor of '2' was selected. Images from the following sections above the orbito-meatal line were obtained (each being a composite of three consecutive slices):

L-1 – Section cut at 19.94 mm above the orbito-meatal line.

L-2 – Section cut at 29.92 mm above the orbito-meatal line (just above section L-1).

L-3 – Section cut at 49.86 mm above the orbito-meatal line (03 pixels above image L-2).

L-4 – Section cut at 69.81 mm above the orbito-meatal line (03 pixels above image L-3).

Semiquantitative Analysis: For quantification, average perfusion per pixel in each region of interest (ROI) in the image being studied was compared with the average perfusion per pixel in the whole image (with all its constituting slices). Thus the average perfusion of each region was obtained as a percentage of the perfusion per pixel in all the three slices. The ROIs drawn were geometric, placed automatically by the system software and allowed the image selected to be rotated / or adjusted to cater for any malalignment.

L-1 image/section was studied through 4 ROIs (02 on the right and two on the left side), L-2 image/section was studied through 16 ROIs (08 on each side), L-3 image/section was studied through another 16 ROIs (08 on each side), and finally L-4 image/section was also studied on a similar pattern with a total of 16 ROIs (08 on each side). Thus, for each subject 52 ROIs were analysed (26 on each side) and the results tabulated.

For an average sized brain, 10 to 14 transaxial images / sections (each three pixels thick) were obtained and the third, fourth, sixth and eighth of these was selected for analysis. Coronal and sagittal images were not studied and the quantification software defaults were minimally altered to keep the results consistent and to aid in their reproducibility.

Statistical Analysis: The data obtained, after tabulation, was statistically analysed. Groups 1 and 2 were compared in respect to age, smoking index (SI), group-wise distribution of abnormal (hypo- or hyper-perfused) ROIs, and subject-wise distribution of abnormal ROIs (hypo- or hyper-perfused ROIs per patient). Mean values, standard deviations and minimum and maximum values found in the above data sets were used for comparative analysis of the chronic tobacco users and non-tobacco users.

The controls (Group 1) were used as the reference for normality, to evaluate the data acquired from all the subjects enrolled in the study. Values / ratios from the various ROIs studied were compared with the corresponding values from Group 1 and a difference of more than + 2 SD (standard deviation) was considered significant. The ROIs were thus classified into normal, hypo-perfused and hyper-perfused. The abnormal ROIs were counted per subject, and their number and percentages per group calculated to statistically determine if the changes observed amongst groups were significant.

P-values, in each case, were calculated employing the χ^2 (chi-squared) test. 2x2 Contingency table was found ideal for computation of χ^2 (chi-squared) values for the study, and the comparison of data obtained for hypo- and hyper-perfused regions of interest (ROIs), from Groups 1 and 2.

Microsoft Excel© was used to process, tabulate and mathematically and statistically evaluate the results obtained from semi-quantitative estimation of cerebral perfusion. All tables and graphs used in the study were also generated through the same software.

RESULTS

The study focused on a group-wise examination of cerebral perfusion patterns in the subjects enrolled for the study. Group-wise semiquantitative evaluation of cerebral perfusion scans of the subjects was carried out and the results recorded.

Semiquantitative Analysis: Group-wise Results – For the semiquantitative analysis, a normal database was created from cerebral perfusion values of the 20 subjects from Group 1. A graphical evaluation of this data, through an analysis of actually acquired whole slice counts (Figure 1) and normalised counts in five randomly selected ROIs, showed it to be normally distributed.

Mean percentage uptake of the radiotracer or percentage perfusion values, and + 2 standard deviation (SD) values in respect of each ROI, for Group 1, were used to evaluate ROIs from all subjects and to classify them into normal and abnormal (hypo-perfused or hyper-perfused) types. An ROI was declared abnormal if its percentage perfusion ratio showed a difference greater than + 2 SD of the perfusion value for that particular slice in the normal control group.

Areas of abnormal perfusion were detected in both groups (controls and chronic tobacco users) but the incidence of hypoperfusion and hyperperfusion showed large variation, both in relation to the percentage of ROIs of each type and their occurrence ratio.

Table 1 shows the group-wise break-up of the total number of ROIs assessed, and the number found abnormal. The percentage of ROIs found abnormal (hypoperfused or hyperperfused) in each group is also shown. Group 1 showed 15 hypoperfused and 27 hyperperfused ROIs out of a total of 1040 ROIs assessed (1.44 % and 2.60 % respectively), while Group 2 showed 164 hypoperfused and 138 hyperperfused ROIs out of the 2496 ROIs assessed (6.57 % and 5.53 % respectively), $P < 0.001$.

Group 1 (Normal Controls): Group 1 contained 20 non-tobacco users, who had Smoking Index (SI) values of less than 100. The perfusion changes in the ROIs studied were minimal in this group. A total of 1040 ROIs were evaluated semi-quantitatively, relative to the reference data. The results obtained in these subjects showed that the number of hypoperfused ROIs is 15 (7 on the right and 8 on the left side) and that of hyperperfused ROIs is 27 (15 on the right and 12 on the left side).

Group 2 (Chronic Tobacco Users): Group 2 contained 48 chronic tobacco users, who had Smoking Index (SI) values of 100 or higher. These subjects showed the greatest variation from the normal in respect of perfusion changes in the ROIs studied. A total of 2496 ROIs were evaluated semi-quantitatively, relative to the reference data from Group 1. Section-wise semiquantitative analysis of cerebral perfusion in these subjects showed that the number of hypoperfused ROIs in the group is 164 (91 on the right and 73 on the left side) and that of hyperperfused ROIs is 138 (54 on the right and

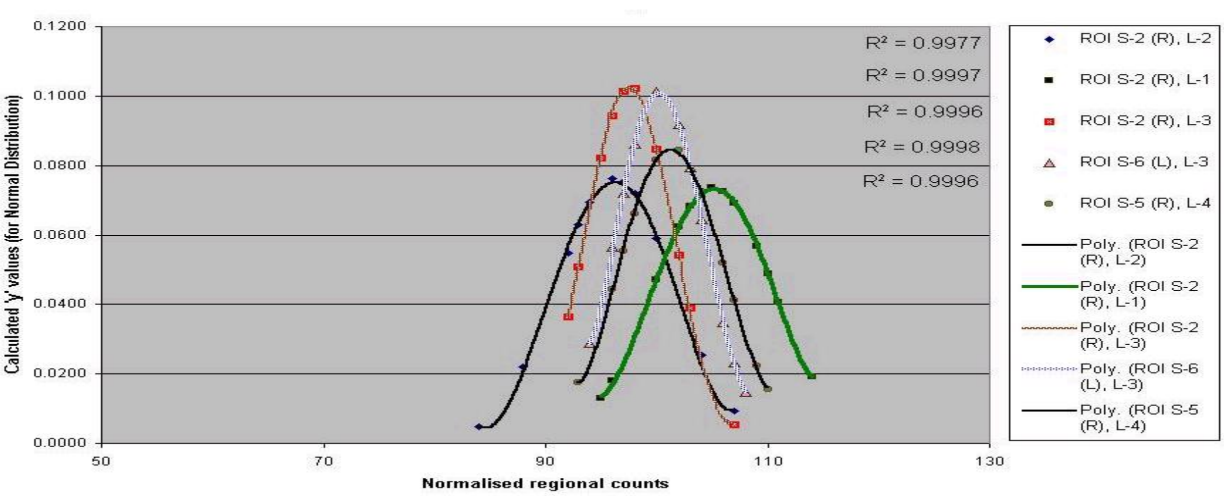

Fig. 1: Distribution of average acquired whole slice counts (Group 1)

Table-1: Group-wise Distribution of Abnormal (Hypo- or Hyper-perfused) ROIs

Group	Number of ROIs assessed	Hypoperfused ROIs	% age	Hyperperfused ROIs	% age
1 (Controls)	1040	15	1.44	27	2.60
2 (Chronic tobacco users)	2496	164	6.57	138	5.53

$P < 0.001$

Table-2: Group-wise Variation in Mean Counts Acquired per Subject in the Slices studied after Injection of 740 MBq (20 mCi) Dose

Group	Average Counts L-1 (a)	Average Counts L-2 (b)	Average Counts L-3 (c)	Average Counts L-4 (d)	Total Average Counts (a + b + c + d)
1 (Controls)	32.15	36.50	38.65	37.55	144.85
2 (Chronic tobacco users)	25.17	29.02	32.02	31.02	117.23

84 on the left side).

Group-wise Break-down of Actual Acquired Counts: As percentage ratios were used to assess cerebral perfusion in the study, it was thought appropriate to correlate the observations with actually acquired counts from the subjects studied. Acquired counts or radiotracer uptake is often used as an indicator of actual tissue perfusion. Acquired average whole slice counts and cerebellar counts were compiled group-wise (Table 2). It was found that the acquired counts were higher in the normal controls (Group 1) when compared with the chronic tobacco users (Group 2). Total average counts, for the control group (Group 1), from all the four slices were 144.85; while for the tobacco users (Group 2), the value was 117.23. The same trend persisted in all the four inidividual slices evaluated (L-1, L-2, L-3, and L-4).

DISCUSSION

This study focused on semi-quantitative estimation of cerebral perfusion and evaluation of changes observed in chronic tobacco users vis-à-vis non-tobacco users. Though there is material available on acute effects of tobacco and nicotine administration on cerebral circulation[15-17], its relationship with chronic tobacco use has not been studied much. A few studies have employed Xe-133[18,19] and other CBF measurement techniques[20] in the past but hardly any have evaluated the changes in cerebral perfusion in chronic tobacco users utilising HMPAO-SPECT, a technique that has frequently been used in other similar studies and is much easier to perform in an average nuclear medicine laboratory.

There was a decrease in the ratio between the hyperperfused and hypoperfused ROIs from the control group (Group 1) to the chronic tobacco users (Group 2) but unexpectedly increase in both hyperperfused and hypoperfused ROIs was seen in chronic tobacco users (Group 2) vis-à-vis non-tobacco users (Group 1).

The ratio between hyperperfused and hypoperfused ROIs was used for the reason that the significant increase in the number of hyperperfused ROIs in smokers may be attributed to the reactive circulatory changes (hyperperfusion) in the brain caused by a chronic nicotine use-induced hypoperfusion in chronic tobacco users. A similar explanation has been proposed by Quirce, et al[21]. in their study, where on semiquantitative assessment of cerebral blood flow in Type I diabetic patients, hyperperfused regions were found in the brain, in addition to hypoperfused regions. Another reason may be the technique used for semi-quantitative estimation, which relies on comparison of different ROIs in the same slice, both ipsilaterally and contralaterally and which may show some areas as hyperperfused when perfusion in the whole slice is non-uniform and includes suboptimally perfused regions. This formed the basis of comparison of the results obtained from the semi-quantitation software, with the actually acquired counts from various brain slices studied for cross-checking the results obtained. A distant possibility may be the acute changes in cerebral circulation caused by recent tobacco use, as some subjects due to their urge for nicotine and / or cigarettes (or Naswaar) could not desist from tobacco use for the recommended period and the presence of some isolated changes due to acute effects of nicotine, emanating from smoking within the preceding 3 to 24 hours of the scintigraphic assessment cannot be totally ruled out. Otherwise, a period of 3 hours has been suggested as enough for elimination of acute effects of tobacco[22,23].

Not losing sight of the aforementioned, there is still an obvious increase in the number of hypoperfused ROIs in Group 2 (chronic tobacco users) compared to Group 1 (normal controls). This increase in hypoperfused ROIs is much more than the increase in hyperperfused ROIs, as is obvious from the group-wise breakdown of hyperperfused and hypoperfused ROIs (Table 1). More studies would be required to unequivocally establish a cause of hyperperfusion, reactive or otherwise, observed in the cerebral circulation in chronic tobacco users.

Use of Average Counts Acquired for Group-wise Perfusion Analysis –

Hyperperfusion or hypoperfusion in a nuclear medicine study can be commented upon – in addition to other recommended criteria – through a critical analysis of radiotracer uptake. The counts acquired are a valuable indicator in this respect. In this study, as is highlighted above and in Table 2, there exists a definite trend between the groups, of a decreasing uptake while comparing Group 1 with Group 2. The average counts acquired, from each of the four 3 pixel-thick sections evaluated show this trend. As all the subjects scanned were injected 740 MBq (20 mCi) of activity under similar conditions, this is a direct indicator of decreased cerebral perfusion amongst the chronic tobacco users, and may alone suffice as a reasonable semi-quantitative measure of cerebral perfusion. It also supports the authors' decision of disregarding the cerebellum as a reference region for comparison of perfusion in other areas, as abnormities in cerebellar perfusion in chronic tobacco users appeared probable in the presence of similar findings in other parts of the brain. The overall findings presented in this study agree with the findings from previous published studies[24,25].

Brain Perfusion Changes and Smoking Index / Extent of Tobacco Use – This study shows a significant difference ($P < 0.001$) in cerebral perfusion in the control group (Group 1) and chronic tobacco users (Group 2). Kubota et al.[26] also documented that the rCBF in smokers was significantly lower than the non-smokers, concluding that acute effects and accumulating chronic effects of smoking on CBF were different. They observed that effects of smoking found by Skinhøj et al[27] and Miyazak[28] (who demonstrated an increase in CBF) and those found by Wechsler[29] (who observed that the CBF remained constant) seemed to be pharmacological effects of nicotine and other compounds in cigarette because rCBF in these studies was compared prior and immediately after smoking. The investigators attributed, rCBF decreases observed, to a higher prevalence of atherosclerosis, which produced vascular narrowing and increased resistance, resulting in CBF decraese.

Findings in this study are somewhat similar to a continuous sample study[30] of 40 asymptomatic individuals (20 smokers and 20 non-smokers), where the researchers, through simple regression analysis demonstrated a significant negative correlation of smoking index with CBF in whole brain, the right parietal cortex, the right occipital cortex and the left parietal cortex.

Variation in Cerebral Perfusion / rCBF with Age – Although the average age of chronic tobacco users (Group 2) enrolled in this study is higher compared to the control group (Group 1), the difference is not a handicap in satisfactory scientific analysis, in light of previous research on the subject, which shows that the greatest decline in cerebral blood flow occurs after the sixth decade, and marked differences are not found among the lower age groups[31-34].

Even if the age difference found amongst the various groups of subjects is taken into account, it is observed that the older volunteer subjects from Group 2 match well with their younger counterparts in the same group. There is no marked change in cerebral perfusion patterns observed in the older sub-group when compared with the younger sub-group. For instance, the only older Group 2 subject with a comparable SI vis-à-vis the younger age sub-group (a 57 year old male with a SI of 260) had a hypoperfused to hyperperfused ROI ratio of 1:4, which was in fact better than his closest match in the younger sub-group (a 31 year old male with a SI of 230), who had a ratio of 2:4.

An important fact to be kept in mind is that the average perfusion of each region was measured relative to the perfusion in the whole slice, or the relative blood flow in various ROIs was expressed as a percentage of the perfusion / average count obtained per pixel. This may give a normal result if the relative uptake / count ratios between various regions are preserved and the semi-quantitative picture may be normal. A subjective assessment in such cases may be helpful and an experienced observer may be able to detect hyperperfusion / hypoperfusion, specially if it is spread over a moderately large region of the cortex, and more significantly if it does not favourably match with the contra-lateral side.

Thus, the deterioration in brain perfusion, with age, which becomes extremely significant in the sixth decade, may to some extent affect a study of factors influencing cerebral perfusion but is not very relevant to the findings in this study because of the presence of obvious perfusion differences, which cannot be explained on the basis of age only. This is further supported by the fact that the average age difference in Group 1 (normal controls) and Group 2 (chronic tobacco users) is only eight years whereas the difference in the number of hypoperfused ROIs is striking (four fold), as is obvious from the statistical significance of the data ($P < 0.001$).

CONCLUSION

This study found significantly decreased overall cerebral perfusion in chronic tobacco users when compared with the non-tobacco users, through semi-quantitative scintigraphic assessment employing Tc-99m HMPAO SPECT.

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