Multimodal imaging of the aging brain: Baseline findings of the LoCARPoN study

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ABSTRACT

We quantified and investigated multimodal brain MRI measures in the LoCARPoN Study due to lack of normative data among Indians. A total of 401 participants (aged 50–88 years) without stroke or dementia completed MRI investigation. We assessed 31 brain measures in total using four brain MRI modalities, including macrostructural (global & lobar volumes, white matter hyperintensities [WMHs]), microstructural (global and tract-specific white matter fractional anisotropy [WM-FA] and mean diffusivity [MD]) and perfusion measures (global and lobar cerebral blood flow [CBF]). The absolute brain volumes of males were significantly larger than those of females, but such differences were relatively small (<1.2% of intracranial volume). With increasing age, lower macrostructural brain volumes, lower WM-FA, greater WMHs, higher WM-MD were found (P = 0.00018, Bonferroni threshold). Perfusion measures did not show significant differences with increasing age. Hippocampal volume showed the greatest association with age, with a reduction of approximately 0.48%/year. This preliminary study augments and provides insight into multimodal brain measures during the nascent stages of aging among the Indian population (South Asian ethnicity). Our findings establish the groundwork for future hypothetical testing studies.

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1. Introduction

With the world's demography rapidly shifting to an era of population aging, it is anticipated that 80% of older adults will live in low- and middle-income countries by 2050 [1]. India is set to observe this demographic trend at a faster rate [2]. In addition to the predominant occurrence of neurodegenerative diseases (such as stroke and dementia) there is a burden of limited interventional strategies. Thus, the paradigm shift toward prevention unquestionably underscores the importance of identifying and addressing the underlying subtle pathological changes in the brain before clinical symptoms manifest. Anatomical and physiological brain parameters such as morphology, vasculature and function have been quantified in several population-based studies [3–5], using different magnetic resonance imaging (MRI) sequences. These studies suggest multiple mixed brain pathologies may be instrumental in

the development of future neurodegenerative diseases, including loss of brain volume, increase in WMHs, and decrease in WM integrity and/or cerebral hypoperfusion [6,7]. In general, only a few cohorts incorporated detailed measures [8], whereas most focused on limited brain outcomes (such as brain volume and WMHs), in older adults (>60 years), including adults with dementia. Hence, it is imperative to understand the non-pathological aging brain with diverse heterogeneous approaches using multimodal brain MRI measures [5]. Predominantly such cohort studies are reported from European [8,9], American [10,11] and/or East-Asian [12] populations. These studies demonstrated variations in results among different ethnic groups [13,14]. In this context, to augment the findings in understudied Indians, the Longitudinal Cognition and Aging Research on Population of the National capital region (LOCARPoN) Study aimed to establish an integrated brain & cognitive aging database to develop Indian-specific risk prediction models. The present study is the first to report normative data of multimodal brain MRI measures in an Indian (South Asian descent) cohort of community-dwelling adults aged 50 years and above, using automated image analysis. Further, we examined the effects of age and sex on comprehensive measures of brain MRI markers including global, lobar and subcortical macrostructural; microstructural and perfusion measures.

2. Material and methods

2.1. Study population

This sub-study is embedded within the epidemiological framework of the LoCARPoN Study, a large population-based cohort study from North India. The LoCARPoN cohort profile and baseline results have already been published, and for additional information refer to the report [15]. The study was approved by the Institutional Ethical Committee at All India Institute of Medical Sciences (AIIMS), New Delhi, India. Written informed consents were obtained separately for study participation and MRI investigation. Since, October 2015, participants aged 50 years and above living in Vasant Kunj, New Delhi (urban population), were invited to participate in the LoCARPoN study.

2.1.1. Analytic sample

A total of 450 participants with complete MRI investigations were screened for eligibility. The reasons for 49 exclusion were: acquisition artifacts (n = 12), severe gliosis (n = 10), stroke (n = 9), >2 missing MRI sequences (n = 4); multiple calcified granuloma (n = 3), chronic hematoma (n = 2), meningioma (n = 2), arachnoid cyst (n = 2), macroadenoma (n = 1); and missing medical site data (n = 3). A total of 405 scans were obtained. Following the quality control (QC) protocol, in the pre-processing phase, MRI sequences were checked for completeness of data; sequences with incomplete data were excluded- Fluid-attenuated inversion recovery (FLAIR) (n = 1), Arterial spin-labeling (ASL) (n = 3), and Diffusion tensor imaging (DTI) (n = 9). After the post-processing phase, visual and outlier assessment resulted in the exclusion of 3D T1-weighted structural (3D T1) (n = 1), Subcortical (n = 1), DTI (n = 7), ASL (n = 10) sequences. After QC, participants with complete 3D T1 & FLAIR (n = 401), Sub-cortical (n = 400), ASL (n = 388) and DTI (n = 385) sequences were included for final analysis.

2.2. MRI acquisition parameters

MR was performed using GE 1.5 T MR scanner (Optima MR450w, General Electric Medical Systems, Milwaukee, Wisconsin, USA) with a 12-channel head coil located at AIIMS, New Delhi, India. The protocol included the following four brain scans (i) 3D T1 images were acquired in the axial plane (repetition time [TR] = 8.4 ms, echo time [TE] = 3.2 ms, imaging time [TI] = 500 ms, 256 × 256 mm2 field of view [FOV], 256 × 256 matrix size, 176 slices, and 1 mm thick); (ii) 3D T2-weighted FLAIR images were acquired in axial plane (TR = 5,500 ms, TE = 96.4 ms, TI = 1,609 ms, 256 × 256 mm2 FOV, 256 X 256 matrix size, 192 slices, and 1.6 mm thick); (iii) 2D DTI shell acquisition images were acquired (TR = 10,983 ms, TE = 76.2 ms, 256 × 256 mm2 FOV, 50 (31volumes) slices, 128 × 128 matrix size, and 3 mm thick); (iv) (iv) 3D pseudo-continuous ASL images were included (TR = 5,206 ms, TE = 11.5 ms, 518 × 8 mm2 FOV, 120 slices, 256 X 256 matrix size, and 3 mm thick) from the LoCARPoN Study MRI protocol [15]. In particular, ASL sequence was optimized with an increase in post-labelling delay (PLD- 2025 ms) to allow more time for blood to reach the brain tissue, as proposed for older adult population [3]. The total scan duration for the above mentioned MR imaging protocol was approximately 22 minutes. A neuro-radiologist examined each MRI for neurologic abnormalities.

2.2.1. Brain MRI processing pipeline and analysis

Image reconstruction was performed on the scanner using standard operations. All the brain MR image data were received in Digital Imaging and Communications in Medicine (DICOM) format. Analysis of brain imaging data was performed using three softwares namely **FSL V5.0.11** (Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library, https://www.fmrib.ox.ac.uk/fsl) [16]; **SPM8** (Statistical Parametric Mapping 8 software, Welcome Trust Centre for Neuroimaging, FIL, London, UK, https://www.fil.ion.ucl.ac.uk/spm/) [17]; and **AFNI Version_19.0.26** (Analysis of Functional NeuroImages software by NIH) [18]. The initial pre-processing steps included data organization of raw data for each participant in separate folders, incomplete/missing images were not processed further. Next, image conversion from DICOM to Neuroimaging Informatics Technology Initiative (NIfTI) format was done using MRIcron software [19]. The registration and processing steps for individual modalities were performed. The detailed processing pipeline is explained in Supplementary Information (SI) and presented as flowchart in SI Fig. 1. Each brain imaging modality was processed separately as employed by most of the population-based studies [9,10,20]. Additionally, 10% of the data were cross-checked at every pre- and post-processing step by an experienced neuroradiologist

blinded by participants' clinical characteristics as part of quality check. A summary of Brain MRI Processing Pipeline is shown in Fig. 1. 3D T1 and FLAIR images were used for macrostructural brain analysis. For **3D-T1 Processing,** brain extraction was performed using Brain Extraction Tool (BET) in the FSL. Visual quality checks for all mask images were carried out in FSLeyes. Manual editing was done to correct unwanted/cut voxels (n = 34). Next, spatial normalization was performed using the 3dQwarp program in AFNI [20], followed by **segmentation**, using FAST (FMRIB's Automated Segmentation Tool) [21] to assign tissue type into CSF, GM and WM. Individual GM lobes (frontal, occipital, parietal, temporal) were segmented with standard atlas; 2 mm resolution version of MNI152 (Montreal Neurological Imaging Template) was used as a template for the reference space that is in line with other aging cohort studies [20]. For subcortical structures (striatum, hippocampus), FIRST (FMRIB's Integrated Registration and Segmentation Tool) [22] was used. Lesion Segmentation Toolbox (LST) in the SPM8 software was implemented in MATLAB 14 [7], and probability maps of WMHs were obtained for **FLAIR processing.** In addition, visual rating of WMHs was carried out with Fazekas Scale, on a scale of 0–3 [23]. For, **DTI processing** correction for eddy currents and motion artifacts was performed out using the FMRIB Diffusion Toolbox (FSL, Oxford, UK). Corrected DTI images were fed into the DTI fitting tool DTIFIT (FSL Software) to create DTI-derived outputs such as FA and MD, based on tract-based spatial statistics (TBSS) [24]. For **Tract-based analysis,** 3D DTI cerebral WM tract atlas developed at John Hopkins University (JHU) [25] was used for six major WM tracts (Anterior Thalamic Radiation [ATR], Cingulate Gyrus [CG], Forceps Minor [FM], Inferior Fronto-Occipital Fasciculus [IFO], Superior Longitudinal Fasciculus [SLF], and Uncinate Fasciculus [UF]) because of their relevance in aging study [26,27]. Quantification of cerebral hemodynamics using a variational Bayesian approach, as implemented in FSL v5.10 was used for **ASL processing** (BASIL; oxford_asl tool) [28]. Calibration of ASL images to obtain CBF maps was performed using a modified Buxton model [3]. Seven perfusion measures, namely, global-CBF, GM-CBF, WM-CBF, and lobe-wise GM-CBF, were selected because they have been implicated in dementia [29,30]. Each brain modality image processing pipeline is shown in SI Figures 2–4. A total of 31 post-processing variables from different modalities were selected for final analysis.

2.3. Statistical analyses

For brain MRI measures, the intracranial volume (ICV) was the sum of GM, total WM and CSF. All the values for brain volume are presented as absolute or as ICV-adjusted (millilitres, ml) to facilitate comparison with other cohort studies. We adjusted macrostructural volumetric measures TBV, GM, WM, lobar and sub-cortical volumes with ICV. The sum of GM and total WM was total brain volume (TBV). The WMHs volume was natural log-transformed owing to the skewed distribution (resulting in negative values for volumes<1 ml). The NAWM was derived by subtracting WMH volume from total WM vol-

ume. The initial analyses did not show any clinical differences between right and left brain measurements, hence, volumes of both sides were summed for macrostructural data, and the average was taken for perfusion data analyses. For each tract, the median FA and MD of the right and left tracts were recorded. We did not adjust for ICV in the microstructural and perfusion measures, as such measures are independent of ICV. Data quality checks and cleaning were performed for all brain measures, including outlier analysis which was part of MRI data QC process (detailed information is provided in SI). Descriptive statistics of the variables are represented as mean with standard deviation for continuous variables and as total count and percentage for categorical variables. Sex differences were assessed using an unpaired *t*-test and a chi-square test (all $P < 0.05$). We evaluated the key assumptions for linear regression before running the model, namely, linear relationship and normality. The relationship between age and all brain measures was evaluated using a linear regression model. To control for inter-individual variability across the study population, the model was adjusted for sex and ICV. Age was included as a covariate in all statistical analyses to control for its potential confounding effect on sex differences in brain measures. For all regression model analysis, we applied a Bonferroni correction for multiple comparisons across 270 independent tests, accepting a strict significance threshold of $P = 0.00018$. This dataset has been subjected to multiple tests that are not part of the present sub-study; hence, a P-value of 0.00018 was considered to indicate a statistically significant difference. For graphic description, scatterplots were plotted and superimposed with a regression line. All statistical analyses were conducted with SAS 12 (SAS Institute, Cary, North Carolina), STATA 15 (StataCorp LLC, College Station, TX), SPSS Statistics 20 and FSLv5.1.

2.4. Data sharing statement

Data from the LoCARPoN Study is available through a formal request to Prof. Kameshwar Prasad [drkameshwarprasad@gmail.com]. Due to confidentiality agreements, data can only be made available to bona fide researchers subject to a non-disclosure agreement. Details of how to request access are available on the study website (https://www.aiimscohortstudy.com or email: aiimscohortstudy@gmail.com). The codes used in the study were the same as stated in the software's user guide (FSL, SPM, AFNI).

3. Results

3.1. Characteristics of the study population

The mean age of 401 LoCARPoN Study participants was 63.69 ± 8.51 years (range, 50–88), and 46.4% were females. The flowchart for the recruitment of participants for MRI is shown in SI Figure 5. On average female participants were younger than their male counterparts (62.12 ± 7.95 vs 65.05 ± 8.75 years, $P < 0.001$). Approximately 76% of the participants were<70 years and only 5% were>80 years.
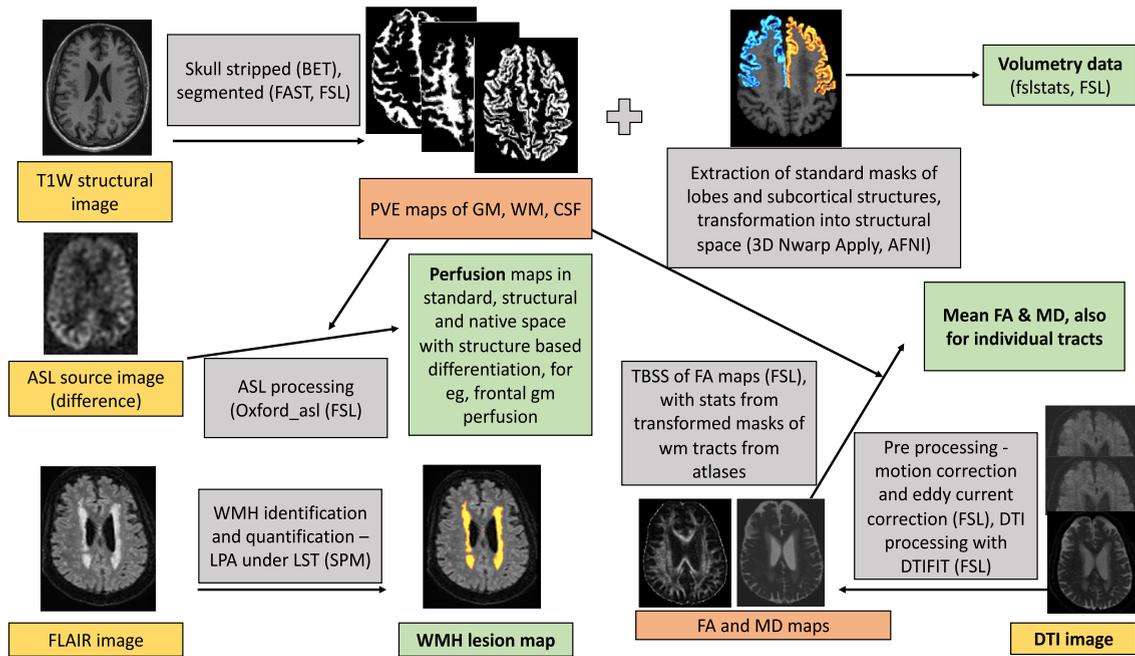
Fig. 1. Summary of Brain MRI Processing Pipeline.

3.2. Sex effect on multimodal brain MRI measures

On average, the mean ICV for females was 144 ml less than that for males [1307.62 ml vs 1163.31 ml; $P < 0.001$]. Compared to females, males had relatively larger absolute macrostructural volumes of all tissue types (all $P < 0.001$; WMHs p = 0.016; hippocampus $P = 0.0009$). The sex-stratified mean ± SD for the absolute brain measures are presented in Table 1, and further ICV-adjusted sex differences are present for macrostructural brain measures (Table 2). Overall, the magnitude of differences after ICV adjustment was relatively small (generally < 1.2% of ICV). The prevalence of WMHs was found to be 48.1% as per Fazekas Scale (193/401). Males consistently had slightly larger WMHs volumes than females ($P = 0.02$). SI Table 1 shows the median of WMH volume for each Fazekas rating. The scatterplots in Fig. 2 (a, b and c) show linear associations between ICV-adjusted brain measures and age. For microstructural brain measures, mean FA ranged from 0.37 to 0.52 and MD from 0.71 to 0.84 ($\times 10^{-3}$ mm/s^2); sex-related differences were statistically non-significant, except for FA of anterior thalamic radiation ($P < 0.001$) and uncinate fasciculus FA ($P = 0.03$); MD of white matter ($P < 0.05$), inferior fronto-occipital fasciculus ($P = 0.01$) and superior longitudinal fasciculus ($P < 0.05$) tracts. The global CBF was found to be 41.51 (SD 5.44) ml/100 g/min. Females had higher CBF values than males (all ($P < 0.001$, WM CBF ($P < 0.02$, except frontal lobe ($P = 0.447$).

3.3. Age effect on multimodal brain MRI measures

A significant negative association was found between ICV and advancing age after adjusting for sex with a decrease of 2.47 ml/year of age ($P = 0.00018$). Linear cross-sectional estimates with increasing age are presented in Table 3. No significant quadratic term was found for brain measures. For macrostructural brain measures and age, model adjusted for sex and ICV showed significant negative association ($P = 0.00018$) for the annual brain volume differences with age, except for frontal lobe GM volume ($P = 0.015$); the following results were observed for atrophy rates (all $P = 0.00018$), highest was for hippocampus 0.48%/year, higher in NAWM (0.25%/year) compared to GM (0.20%/year). For lobar brain measurements, differences in GM was relatively high in occipital (0.38%/year) and temporal lobes (0.28%/year) and subtle for frontal lobe (0.07%/year). The annual increase in WMHs and decrease in hippocampal and striatal volumes with age were significant (all $P = 0.00018$). Furthermore, age was significantly associated with both lower FA and higher MD in all white matter tracts (all $P = 0.00018$); the overall annual decrease in white matter FA was 0.33%/year and increase in MD was 0.18%/year. The linear relationship with age was most prominent in the fiber tract groups cingulate gyrus and forceps minor (FA 0.45%/year and 0.49%/year respectively; MD 0.20% and 0.24%/year respectively). Perfusion brain measures showed a non-significant positive association with global as well as regional CBF measures with an increase in age. Further analysis revealed that for each additional year of age, perfusion measures increased only by 0.12 ml/100 g/min, which was a small change when compared to the high variability of ASL measures itself (Global CBF SD = 5.44 ml/100 mg/min). With advancing age, males showed more brain volume reduction when compared to females (SI Table 3).

Hence, the findings of augmented perfusion, that is an increase in perfusion with age implies no significant

Table 1
Summary of demographic and brain measures of study participants.

Variables	Participants (N, F/M)	Total N = 401	Female 186 (46.4)	Male 215 (53.6)	P value
Demographics					
Age (years)	401, 186/215	63.69 ± 8.51	62.12 ± 7.95	65.05 ± 8.75	**<0.001**
Age group					**<0.001**
50–59		128 (31.9)	74 (39.8)	54 (25.1)	
60–69		176 (43.8)	79 (42.5)	97 (45.1)	
70–79		75 (18.7)	27 (14.5)	48 (22.3)	
80 and above		22 (5.5)	6 (3.2)	16 (7.4)	
Education (years)	399, 185/215	15.61 ± 3.49	14.83 ± 3.81	16.28 ± 3.04	**<0.001**
Educational Status	399, 185/215				**<0.001**
College		324 (81.2)	145 (78.4)	179 (83.6)	
Higher Secondary Secondary		28 (7.0)	12 (6.4)	16 (7.5)	
Diploma/vocational		18 (4.5)	11 (5.9)	7 (3.2)	
Primary or below primary No formal education		16 (4.0)	6 (3.2)	10 (4.6)	
		11 (2.7)	9 (4.8)	2 (0.9)	
		2 (0.5)	2 (1.1)	0 (0.0)	
MMSE	391, 181/210	27.98 ± 2.33	27.8 ± 2.54	28.14 ± 2.13	0.14
Brain Measures					
Macro-structural Brain Measures (ml)					
Intracranial Volume	401, 186/215	1240.68 ± 117.39	1163.31 ± 91.23	1307.62 ± 94.12	**<0.001**
Gray Matter (GM)	401, 186/215	522.95 ± 45.81	498.75 ± 40.68	543.89 ± 39.26	**<0.001**
Normal Appearing WM	401, 186/215	422.81 ± 47.88	398.51 ± 41.18	443.84 ± 43.15	**<0.001**
WMHs	401, 186/215	−0.55 ± 2.09	−0.93 ± 1.9	−0.4 ± 2.11	**0.02**
Cerebrospinal fluid	401, 186/215	522.95 ± 45.81	264.5 ± 28.07	317.7 ± 34.58	**<0.001**
Fazekas Rating		208 (51.87)	103 (55.37)	105 (46.51)	0.07
0	401, 186/215				
1	401, 186/215	110 (27.43)	48 (25.80)	62 (28.83)	0.49
2	401, 186/215	70 (17.46)	30 (16.12)	40 (18.60)	0.51
3	401, 186/215	13 (3.24)	5 (2.68)	8 (3.72)	0.558
Total Brain Volume	401, 186/215	947.68 ± 90.29	898.8 ± 77.71	990.0 ± 78.39	**<0.001**
Frontal GM Lobe	401, 186/215	156.84 ± 15.66	149.25 ± 14.08	163.41 ± 13.91	**<0.001**
Parietal GM Lobe	401, 186/215	100.99 ± 10.27	96.5 ± 9.28	104.87 ± 9.5	**<0.001**
Occipital GM Lobe	401, 186/215	62.24 ± 6.67	59.31 ± 5.98	64.77 ± 6.2	**<0.001**
Temporal GM Lobe	401, 186/215	99.87 ± 10.34	93.98 ± 8.98	104.97 ± 8.61	**<0.001**
Hippocampus	401, 186/215	3.59 ± 0.41	3.52 ± 0.37	3.65 ± 0.43	**<0.001**
Striatum	401, 186/215	7.7 ± 0.79	7.38 ± 0.73	7.98 ± 0.72	**<0.001**
Micro-structural Brain Measures (MD values 10^{-3} mm/s^2)					
WM FA	385, 177/208	0.45 ± 0.03	0.45 ± 0.03	0.45 ± 0.03	0.49
ATR FA	385, 177/208	0.35 ± 0.02	0.35 ± 0.02	0.36 ± 0.02	**<0.001**
CS FA	385, 177/208	0.4 ± 0.04	0.39 ± 0.04	0.4 ± 0.04	0.26
FM FA	385, 177/208	0.39 ± 0.03	0.39 ± 0.03	0.39 ± 0.03	0.68
IFO FA	385, 177/208	0.4 ± 0.02	0.4 ± 0.02	0.4 ± 0.02	0.79
SLF FA	385, 177/208	0.38 ± 0.02	0.38 ± 0.02	0.38 ± 0.02	0.44
UF FA	385, 177/208	0.37 ± 0.02	0.37 ± 0.02	0.38 ± 0.03	**0.03**
WM MD	385, 177/208	0.77 ± 0.03	0.76 ± 0.03	0.77 ± 0.03	**<0.001**
ATR MD	385, 177/208	0.79 ± 0.03	0.79 ± 0.03	0.79 ± 0.03	0.18
CS MD	385, 177/208	0.78 ± 0.03	0.78 ± 0.03	0.78 ± 0.03	0.16
FM MD	385, 177/208	0.8 ± 0.04	0.8 ± 0.04	0.81 ± 0.04	0.21
IFO MD	385, 177/208	0.81 ± 0.03	0.80 ± 0.03	0.81 ± 0.03	**0.01**
SLF MD	385, 177/208	0.77 ± 0.03	0.76 ± 0.03	0.77 ± 0.03	**0.05**
UF MD	385, 177/208	0.8 ± 0.03	0.8 ± 0.03	0.8 ± 0.03	0.06
Perfusion Brain Measures (ml/100 g/min)					
Global CBF	388, 182/206	41.51 ± 5.44	40.46 ± 4.99	42.69 ± 5.69	**<0.001**
GM CBF	388, 182/206	45.24 ± 6.25	46.68 ± 6.44	43.97 ± 5.79	**<0.001**
WM CBF	388, 182/206	35.75 ± 4.6	36.32 ± 4.88	35.25 ± 4.3	**0.02**
Frontal GM CBF	388, 182/206	46.24 ± 6.52	46.51 ± 6.66	46 ± 6.4	0.44
Parietal GM CBF	388, 182/206	46.08 ± 7.95	49.77 ± 7.88	42.83 ± 6.46	**<0.01**
Occipital GM CBF	388, 182/206	49.49 ± 7.5	51.63 ± 7.71	47.6 ± 6.78	**<0.01**
Temporal GM CBF	388, 182/206	39.87 ± 5.14	41.15 ± 5.33	38.75 ± 4.69	**<0.01**

Notes-Values for MD and CBF were 10^{-3} mm^2/s and ml/100 g/min respectively. ATR = Anterior Thalamic Radiation, CS = Cingulate Gyrus, CBF = Cerebral Blood Flow, FA = Fractional Anisotropy, FM = Forceps Minor, GM = Gray matter, IFO = Inferior Fronto-Occipital Fasciculus, MD = Mean Diffusivity, SLF = Superior longitudinal fasciculus, UF = Uncinate fasciculus WM = White matter, WMHs = White matter hyperintensities. $P < 0.05$, considered significant differences in means between sexes (bold).

change in CBF with increasing age (SI Table 2, shows age-range distribution of perfusion measures). In summary, Bonferroni correction for multiple comparisons (P = 0.00018) showed that all associations remained significant except for perfusion brain measures and frontal GM lobe volume.

Table 2
ICV adjusted volumes for Macrostructural Brain Measures.

	Female (186)		Male (215)		P-Value	††
	Absolute Volume (ml) (M±SD)	ICV adjusted Volume (ml) (M±SD)	Absolute Volume (ml) (M±SD)	ICV adjusted Volume (ml) (M±SD)		
GM	498.75±40.68	526.21±18.03	543.89±39.26	520.13±19.47	<0.001	F>M
NAWM	398.51±41.18	427.53±17.17	443.84±43.15	418.73±19.23	<0.001	F>M
WMH[a]	-0.93±1.9	-0.76±1.8	-0.41±2.11	-0.5±1.6	0.11	NS
TBV	898.8±77.71	955.58±23.67	989.96±78.39	940.84±27.99	<0.001	F>M
Frontal GM	149.25±14.08	158.16±7.56	163.41±13.91	155.7±8	0.001	F>M
Parietal GM	96.5±9.28	101.99±5.69	104.87±9.5	100.11±6.15	0.001	F>M
Occipital GM	59.31±5.98	62.47±4.46	64.77±6.2	62.03±4.79	0.34	NS
Temporal GM	93.98±8.98	99.68±5.65	104.97±8.61	100.03±5.71	0.53	NS
Hippocampus	3.52±0.37	3.64±0.33	3.65±0.43	3.55±0.4	0.02	F>M
Striatum	7.3±0.43	7.5±0.35	7.67±0.49	7.49±0.41	0.76	NS

Notes: Absolute (no colour) and ICV-adjusted volumes (highlighted blue) are reported for comparison, The columns show the comparison of sex-stratified on adjustment with ICV. P-value for differences in means between sexes (bold), †† overall comparison between males and females, GM = Gray matter, NAWM = Normal appearing white matter, NS = Not significant. SD = Standard deviation, WMH White matter hyperintensities. P < 0.05, considered significant differences in means between sexes (bold).

4. Discussion

In this urban community-based cohort of middle-aged and older adults from India, we estimated the linear cross-sectional brain MRI measures for different modalities, namely macrostructural, microstructural, and perfusion. To our knowledge, this is the first study to report normative multimodal brain MRI estimates and examine age-sex differences in comprehensive brain markers for a community representative from the northern part of India (Delhi). To fully reflect non-pathological brain measures, we deliberately limited our exclusion criteria to symptomatic neurological disorders. The study outcomes reveal three core findings. First, age is associated with lower macrostructural volumes and microstructural brain integrity that vary quantitatively according to the brain regions examined. Second, sex differences were generally modest when compared with age-related brain measurement differences. Third, perfusion measures are higher in females than males, however, with increasing age, perfusion measures did not attain statistical significance.

4.1. Effects of sex on multimodal brain measures

Overall brain measure results are comparable with those of other epidemiological studies among multi-ethnic groups from AGES-Reykjavek Study [31], ARIC Study [11], Framingham Study [10], Rotterdam Study [9], Singapore Longitudinal Aging Brain [32], Strong Heart Study [33], UK Biobank Study [34], and Washington Heights/Hamilton Heights Aging Project (WHICAP) [35]. Similar to our findings males had proportionally larger absolute brain volumes than females. This was expected because males have generally larger heads corresponding to their overall larger stature [36]. The magnitude of these sex differences was relatively small in our study (<1.2% ICV). Our data represents slightly lower values than other brain imaging studies that have found sex differences ranging from <2–3% of ICV for brain volumes [35]. We had relatively fewer older adults, such age difference between participants may explain discrepancies in the results. A few of our findings were inconsistent with previous reports, such as, on adjustment with ICV [9,37], male participants in present cohort had proportionally smaller GM and WM volumes, and slightly more WMHs, whereas in the Rotterdam study males had larger volume of WM and fewer WMHs [9]. Adjustment with ICV was performed, in order to facilitate comparison with other cohort studies, however, methodological considerations of brain MRI analysis and age differences may account for these differences. For microstructural measures, slightly higher FA values were found in males compared than in females, which is consistent with the findings of previous studies [34]. Possible differences in head size often attributed to this [38], men
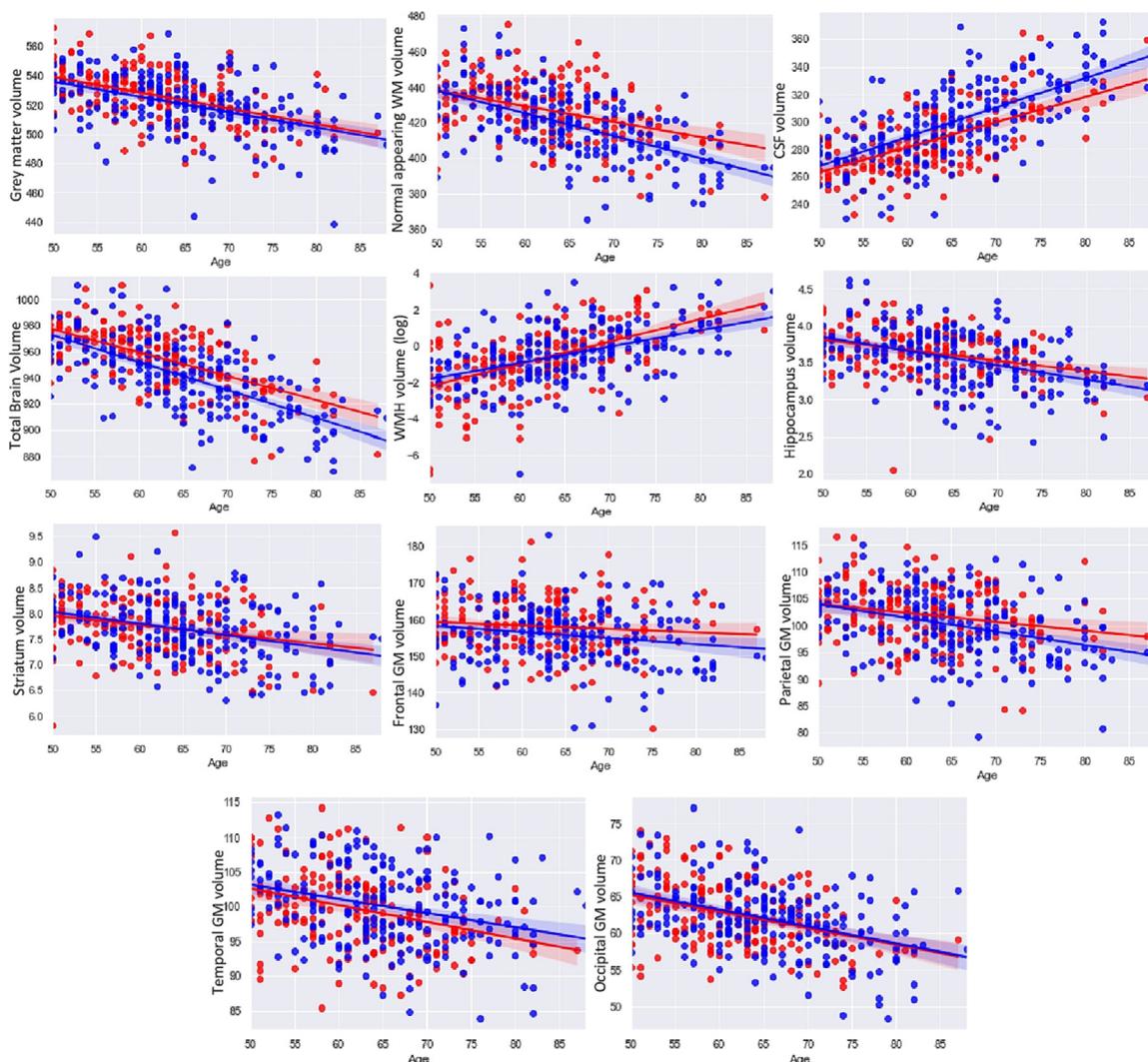
Fig. 2a. Scatter-plots showing ICV-adjusted macrostructural brain measures with age for females (red) and males (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

have higher intra-axonal volume fractions [39], and increased extracellular space [40]. Our findings of relatively high CBF values in females are in accordance with the previously published results [41,3]. Women have higher levels of estrogens and prostacyclin than men, and increasing vascular CO_2 reactivity is likely to account for higher CBF [42]. Correspondingly, men have higher haemoglobin concentrations than women, and oxygen consumption is at similar rate for both; hence, the underlying neurovascular regulatory mechanisms that maintain a normal oxygen supply may lead to increased CBF in women [43]. Our findings on cerebral hemodynamics are comparable to growing evidence from population-based studies using ASL [44,45]. Although slightly lower perfusion estimates were found when compared to studies from the Caucasian population [45,46], nonetheless consistent with

a recent study from the African-American ethnic group who showed similar GM CBF (45.52 ml/100 g/min) in participants aged 63.23 ± 8.28 years, [44]. Recent evidence that hematocrit differences by sex and race/ethnicity can influence the quantification of perfusion estimates from ASL [47]. Individual hematocrit was not measured in this sub-study sample and a fixed T1 blood estimate was used [3] for perfusion quantification. As post-labelling delay (PLD) affects the CBF quantification, evident from a recent study in middle-aged people (>46–65 years) that proposed the optimal PLD should be 2525 ms for most brain regions [48]. This might have additionally resulted in our underestimation CBF values due to PLD of 2025 ms used in the study. Another study by Juttukonda and colleagues [49] proposed a cerebral hemodynamics processing approach with multi-PLDs, which may be considered for future studies.
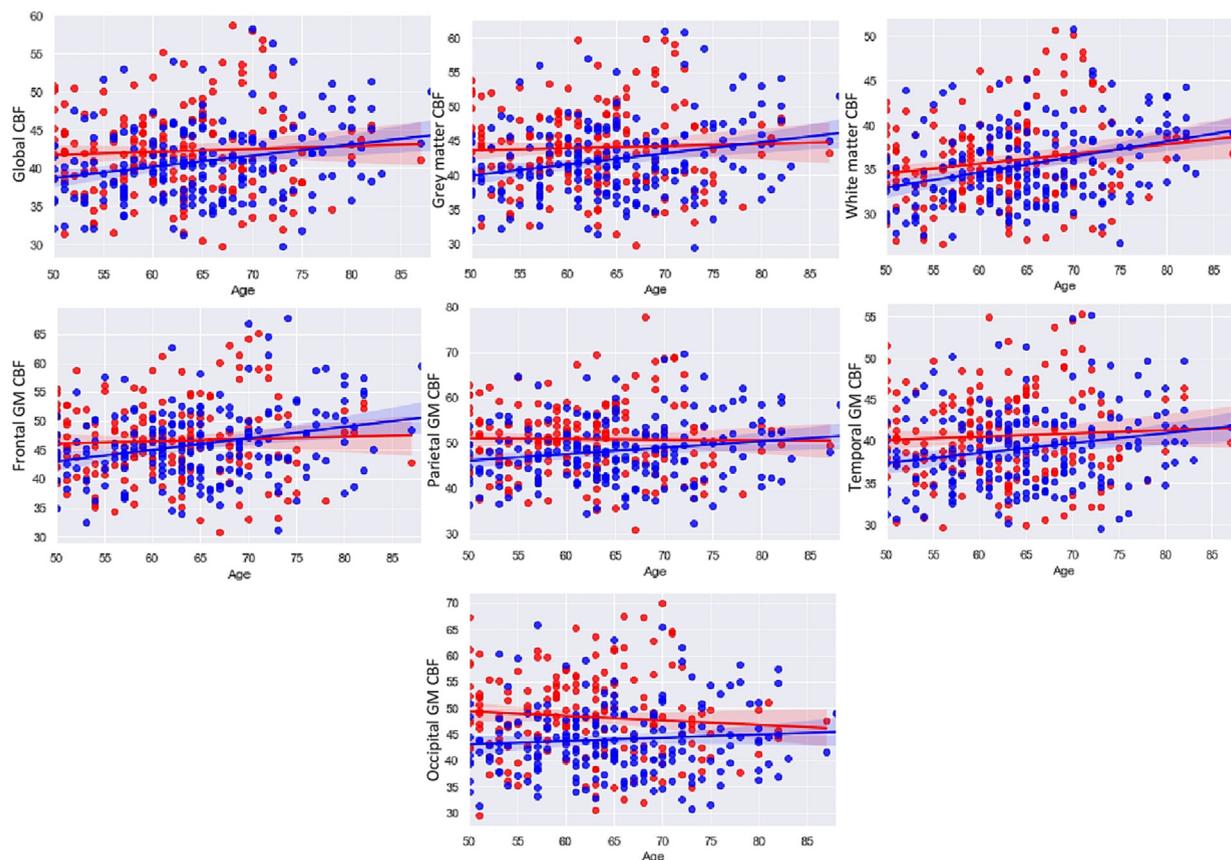
Fig. 2b. Scatter-plots showing ICV adjusted perfusion brain measures with age for females (red) and males (blue).

4.2. Effects of age on multimodal brain measures

Studies have consistently shown increasing age is associated with smaller brain volumes in persons above 60 years of age [10,31], our findings are in line with those of prior studies. In the present cohort, there were age-related differences in the WM volume and GM volumes, with WM volume reducing at a slightly higher rate than GM volume. One possible explanation may be age under consideration, and previous studies included more elderly volunteers [9,31]. Secondly, post-mortem evidence suggests that there is minimal GM decline in healthy older adults [50] and that GM atrophy is more prevalent in those with increased dementia risk [9]. Other studies primarily investigated a broad age range, [51] whereas our focus was on adults aged 50 years and above. This also supports our findings for a linear trend, adding a quadratic term did not improve our model [31]. The rate of change of several brain morphological metrics estimated from our middle-aged to older adults was slightly lower in estimates than in Western [10,11] and East Asian cohorts [32,52]. Inter-ethnic disparities may explain these differences [53] and age, where 75% of our study population was<70 years old. The most extensively reported brain measure in aging research is TBV with an annual percent change (APC) of 0.18–0.88%/yr [54,55], an average of 0.20%/yr was found

our cohort and was mostly observed to be at the lower end when compared with other studies that analysed generally elderly subjects [31,37]. Our cross-sectional APC estimate for hippocampal volume was 0.48%/yr, the most pronounced association with age among all the analysed structures. This is consistent with other population-based studies, which have showed a range of 0.3– 1.5%/yr [51]. A recent study conducted on South Asians living in Singapore with Indian participants also supports our finding of more subcortical atrophy when compared to Chinese and Malays. In contrast, the Chinese and Malays, displayed significantly higher cortical atrophy [53]. Several factors may contribute to these differences, including genetic variation and exposure to environmental and lifestyle factors [56]. Among lobar volumes, the significant age-related difference was highest in the occipital lobe GM with least in the frontal lobe GM volume. There is evidence that prominent cortical thinning around the primary visual cortex resulting in occipital lobe atrophy with age [57]. The annual rates of GM decline ranges from 0.56 to 1.05% in frontal, 0.43 to 0.55% in temporal, 0.21 to 0.90% in parietal, and 0.33 to 0.36% in occipital lobes [51], which was comparable for our participants. Post-mortem studies support the hypothesis that regional vulnerability persists as we age [58], but its precise cause remains unknown [59]. As seen in our data and as noted by others, brain
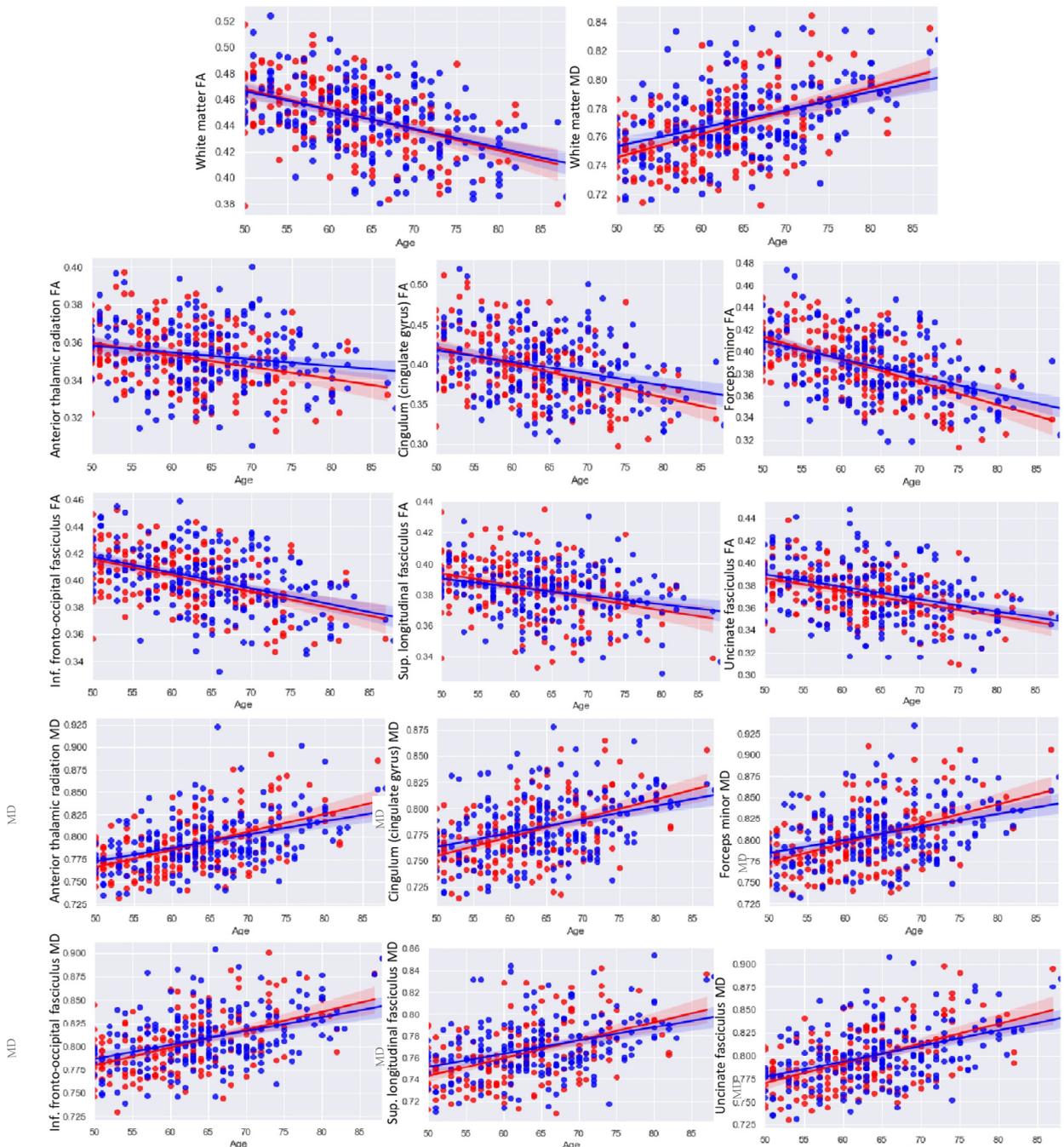
Fig. 2c. Scatter plots showing micro-structural FA & MD brain measures with age for females (red) and males (blue).

structural association with age are heterogeneous [10,35] across individuals in different topographical regions. With advancing age, brain volume decreases more rapidly in males compared to females [60], and our findings elicited similar patterns. In addition, cerebral small vessel diseases, such as WMHs were found to be highly prevalent in our study population, which are common with aging [61]. However, the reported WMHs volume [IQR 0.20–1.65 ml] is lower than expected for the age range studied, and even when compared to 45 years old [IQR 0.4–1.14 ml] [62]. One possible explanation may be our cohort's higher educational attainment; similar findings were found in the Framingham Offspring Study [63].

Table 3
Cross-sectional association between the difference in macrostructural, microstructural and perfusion per year increase of age.

Macrostructural (volume)				Microstructural								Perfusion (CBF)			
					Fractional Anisotropy			Mean Diffusivity					β	% change	P-value
					β	% change	P-value	β	% change	P-value					
Measures	β	% change	P-value	Measures							Measures				
TBV	-1.95	**-0.21**	**<0.0001**	WM	-0.0015	**-0.33**	**<0.0001**	0.0014	**0.18**	**<0.0001**	Global CBF	0.122	0.30	0.0003
GM	-1.05	**-0.20**	**<0.0001**	ATR	-0.0005	**-0.14**	**<0.0001**	0.0017	**0.22**	**<0.0001**	GM CBF	0.133	0.31	0.0003
NAWM	-1.07	**-0.25**	**<0.0001**	CG	-0.0018	**-0.45**	**<0.0001**	0.0015	**0.20**	**<0.0001**	WM CBF	0.158	0.44	0.0002
Frontal GM	-0.61	**-0.07**	**0.0157**	FM	-0.0019	**-0.49**	**<0.0001**	0.0019	**0.24**	**<0.0001**	Frontal GM	0.145	0.32	0.0004
Parietal GM	-0.48	**-0.21**	**<0.0001**	IFO	-0.0012	**-0.30**	**<0.0001**	0.0017	**0.21**	**<0.0001**	Parietal GM	0.112	0.23	0.2637
Occipital GM	-0.33	**-0.38**	**<0.0001**	SLF	-0.0007	**-0.18**	**<0.0001**	0.0014	**0.18**	**<0.0001**	Occipital GM	0.051	0.11	0.0153
Temporal GM	-0.42	**-0.24**	**<0.0001**	UF	-0.0012	**-0.32**	**<0.0001**	0.0019	**0.20**	**<0.0001**	Temporal GM	0.108	0.27	0.0007
Hippocampus	-0.02	**-0.48**	**<0.0001**												
Striatum	**-0.01**	**-0.24**	**<0.0001**												
WMH	0.11		**<0.0001**												

Notes: Regression Model adjusted for Sex, ICV. β Coefficients with (p values = 0.00018) are reported. Coloured and bold types indicate significant results. % change/year: volume difference per year of age based on the linear estimate. ATR = Anterior Thalamic Radiation, CS = Cingulate Gyrus, FM = Forceps Minor, IFO = Inferior Fronto-Occipital Fasciculus, SLF = Superior longitudinal fasciculus, UF = Uncinate fasciculus.

Regarding the global WM integrity, a widespread loss of microstructural organization with increasing age was observed, which is widely established [26]. Prior evidence has shown a negative correlation between FA and age, beginning well before the age of 45–50 years [64], whereas MD is sensitive to white matter changes driven by vascular disease and positively associated with age [65]. As for regional tract-specific vulnerability to aging, mounting evidence suggests that frontal region is more susceptible than a more posterior region, which is consistent with lower WM integrity in the cingulate gyrus and forceps minor tracts, which belong to the prefrontal region [66]. In contrast, to decline in perfusion with aging, our cohort showed a modest positive association, although this was clinically non-significant and safe to be called as preservation [67]. There are few prior findings on augmented perfusion in healthy older adults [68,69] using ASL and SPECT [67]. These results may be attributed to lower number of older participants (>70 years) in the present study. It should be noted that cerebral perfusion is highly variable owing to several physiological parameters, an extensive systematic review identified 58 possible perfusion modifiers, including blood caffeine levels, blood gases, and blood pressure [70]. Additionally, acquisition parameters of the ASL scan may have also resulted in underestimation of the CBF [3,48].

It is important to consider two factors before interpreting our results in the context of published literature. Firstly, it should be noted that the age range under consideration is from 50 to 88 years in stroke and dementia-free participants, unlike other life-span studies or studies specific to elderly persons including those with dementia and/or cognitively impairment [37]. Secondly, there were fewer older adults (n = 22; ≥80 years). Therefore, our findings show a smaller association between these measures.

4.3. Strengths and limitations

The study was embedded within population-based setting. In a limited resource country, such as India, acquisition of multimodal neuroimaging sequences to assess volumetric, WM integrity and perfusion measures with detailed availability of clinical data was available at a single point in time, in contrast to other cohort studies, where MRI investigation was performed a few years after baseline data collection. This may be advantageous for future follow-up studies. Furthermore, robust and automated procedures were used for analysis compared to visual rating scales. This has been attributed to the rapid advancement in brain quantification over the last decade. A number of methodological considerations need attention before interpreting our results within the context of the published literature. With reference to study participants living in the Vasant Kunj, Delhi (Northern part of India), it seems as an ideal location given the relatively homogenous environment with diverse cultural aspects of Indians staying in this region. First, causal inferences could not be drawn from the cross-sectional study design. Therefore, future longitudinal studies are warranted. Second, our cohort of participants only included the urban population which limits the generalizability; for comparison future studies will include participants from rural component of

LoCARPoN Study. However, this study can act as a benchmark to compare future studies in the aging brain. Third, due to the discrepancy in our sample of fewer older adults, the holistic effect of age on brain variables was not well-captured. Including more older individuals or running a post-hoc analysis for middle aged and older adults separately may provide more information in the future. Fourth, we only reported the hippocampus and striatum, as not all individual subcortical structures were included in this study. A detailed discussion on subcortical structures is beyond the scope of this manuscript and is a priority for future studies. Finally, out of the four sequences, it must be noted that ASL sequence may provide variable information when repeated at different time intervals even for the same individual. This variability is introduced due to the sequence as such, along with the noise added by various artifacts such as motion (cases with obvious motion artifacts were excluded, however, it would be impossible to detect minor changes). This can be partly resolved by repeated MRI measurements of such sequences, taking multiple post-label delay in ASL, or normalising the perfusion measures with standard anatomical areas for processing analysis. We assume that our quality control measures followed as reported by other population-based studies, would have minimized the errors.

5. Conclusion

Our study presents preliminary data that delineates normative values for multimodal brain measures among middle-aged and older adults in India. Additionally, our findings provide association of brain measures with age and sex. Future hypothesis-driven studies can be designed based on these baseline results of aging brain.

CRediT authorship contribution statement

Pallavi Nair: Conceptualization, Methodology, Investigation, Software, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Kameshwar Prasad:** Conceptualization, Funding acquisition, Supervision, Methodology, Resources, Formal analysis, Writing – review & editing. **Parthiban Balasundaram:** Software, Resources, Validation, Writing – review & editing. **Deepti Vibha:** Methodology, Resources, Supervision, Writing – review & editing. **Sada Nand Dwivedi:** Methodology, Resources, Supervision, Formal analysis, Writing – review & editing. **Shailesh B. Gaikwad:** Methodology, Software, Resources, Software, Supervision, Writing – review & editing. **Achal K. Srivastava:** Supervision, Resources, Writing – review & editing. **Vivek Verma:** Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbas.2023.100075.

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