Original Article

Establishment of Normative Data for Autonomic Function Tests in Indian Population

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Abstract

Background: Normative data for autonomic function tests (AFT) is not available for Indian population. **Objective:** The aim of the study was to establish normative data in AFT and its correlation with age, gender, and body mass index. **Material and Methods:** The study was done on 254 healthy subjects of age ≥18 years. All AFTs were done in autonomic laboratory at the Department of Neurology, Christian Medical College and Hospital, Ludhiana. Cardiovascular tests (heart rate response to deep breathing, HR changes in Valsalva maneuver and head-up tilt test (HUT)) and quantitative sudomotor axon reflex testing (QSART) were performed in all the subjects. Fifty subjects underwent thermoregulatory sweat test (TST). **Results:** The mean age (SD) of study participants was 43 (16.0) years (range 20–84), and 129 (50.8%) were men. The normative value range (2.5–97.5 percentile) for HR difference, E: I ratio, and Valsalva ratio (VR) was 3.5–47.0, 1.05–1.93, and 1.11–2.64, respectively, for all the subjects. HR difference and E: I ratio showed an significant inverse relation with age (r = -0.623 and r = -0.584, respectively). VR also showed an inverse relation with age (r = -0.575, P =< 0.001), and female had a lower value than male (1.63 vs 1.78, P =< 0.001). In QSART, mean (SD) sweat volume was higher in males 0.630 (0.230) compared to females 0.513 (0.132) for all sites, P < 0.001, and similar trend was noticed for sweat area in TST. **Discussion and Conclusion:** Normative AFT data has been established for Indian population for the first time. The values are comparable to previously published studies.

Keywords: Autonomic function test, cardiovascular, normative data, QSART, thermoregulatory sweat test

Introduction

The autonomic function test (AFT) is a battery of tests devised to study the sympathetic and parasympathetic branches of the autonomic nervous system (ANS). The effect of specific provocative maneuvers on cardiovascular reflexes forms the basis of these tests. Sympathetic activity can be studied by the blood pressure (BP) response to orthostatic testing and Valsalva maneuver (VM). Parasympathetic function can be evaluated by studying the changes in heart rate (HR) during orthostatic testing, VM and deep breathing (DB).[1-5]

There are age and gender differences in the values of autonomic tests. The largest study for normative data in individuals between 10 and 83 years was done in USA, which showed a decrease in cardiovagal function with age. The same study demonstrated gender differences in quantitative sudomotor axon reflex testing (QSART).[6] There is lack of similar data for the Indian subcontinent. Availability of normative data is important to diagnose patients with autonomic disorders. Hence, we aim to establish normative data for the Indian subcontinent and its correlation with age, gender, and body mass index (BMI).[7,8]

Materials and Methods

A total of 254 healthy subjects of aged ≥20 years were recruited in the study during the period from 04-01-2017 till 09-31-2019. Cardiovascular tests (heart rate response to deep breathing (HRDB), HR changes in VM and HUT) and QSART were performed in all the subjects. Fifty subjects underwent thermoregulatory sweat test (TST). They were evenly distributed by age and gender. Participants with any systemic diseases like diabetes mellitus, hypertension, cardiac diseases, or taking medication with effects on the ANS were excluded from the study. The study was approved by institutional ethics committee and written informed consent was taken from each subject. The age and gender distribution of the 254 normal subjects by tests are shown below:

Age-group (males, females): 20–30 (32, 30), 31–40 (41, 39), 41–50 (16, 16), 51–60 (15, 15), 61–70 (15, 15), and ≥71 (10, 10).

All tests were done in the Autonomic laboratory at the Department of Neurology, Christian Medical College and Hospital, Ludhiana. The machines used for recording the AFTs

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were: iVY-Cardiac Trigger Monitor 3000, WR-Test Works™ Analog Interface (WR-Medical Electronics Co), bmeye- Nexfin Monitor Model1 (Bmeye Cardiovascular Intelligence), Tilt table (WR-Medical Electronics Co), Q-SWEAT – Quantitative Sweat Measurement System (WR-Medical Electronics Co), Nexfin HRS, and wrist unit model1. The HR and BP were monitored continuously. Autonomic function testing was done as per standard protocols as follows[9]:

1. HRDB: We recorded BP and HR for 3 min with subject in a resting position. The subjects were then asked to breath maximally at a rate of 6 breaths/min (inspiratory and expiratory cycles of 5 s each), establishing a smooth maximal inspiratory and expiratory rhythm. Eight cycles (deep breaths) were recorded followed by resting BP and HR for 3 min. After a resting period of 3 min, DB cycles were repeated twice and recorded (a total of three times) and the five largest consecutive responses per cycle were read from the computer by the operator, manually placing a cursor over the trace. The average HRDB difference (maximum–minimum) of the five largest consecutive responses in the three sets was derived
2. HR and BP changes in VM**:** Resting recording was done with subject lying in the recumbent position for 3 min preactivation. To proceed with activation; mouthpiece of bugle with an air leak (to ensure an open glottis) was raised towards the volunteer, who was instructed to take a deep breath in. The subject formed a good seal around the mouthpiece and blew into it to maintain a column of mercury at 40 mm Hg, for 15 s. Postactivation, resting recording was taken for another 3 min. The procedure was repeated three times. The Valsalva ratio (VR) was derived from the maximum HR divided by the lowest HR following the VM. Inclusion criteria for an acceptable recording were: (i) expiratory pressure at least 30 mm Hg and maintained for 10 s; (ii) reproducible VM BP curve; and (iii) absence of a flat-top BP curve. The baseline values of BP (systolic BP [SBP], mean arterial BP [MAP], diastolic BP [DBP]) were derived from the average of readings during the stable 30 s before the VM. The amplitude of phase 1 was measured from baseline to peak (I). The reduction of early phase 2 was measured from baseline to the trough of phase 2 (II_e). The magnitude of late phase 2 (II_l) was determined from end of early phase 2 to the beginning of phase 3 (III). The amplitude of phase 3 was measured from the end of late phase 2 to the trough of phase 3 (III). The magnitude of phase 4 was determined as its height above baseline (IV). For the responses, the largest data from a satisfactory expiratory pressure was accepted

   The BP recovery time was calculated for SBP, MAP, and DBP curves as described. Time intervals were then determined for two periods of the maneuver: (i) from the lowest phase 3 amplitude to complete return of SBP, MAP, and DBP to baseline pressure recovery time (PRT 100) and (ii) from the lowest phase 3 amplitude to 50% return of SBP, MAP, and DBP to baseline (PRT 50). The average SBP, MAP, and DBP in each instance was used to determine the baseline
3. HUT: Straps were applied over the upper chest and across knees to secure the subject to the table. Baseline trace recording was done for 10 min with the volunteer resting quietly in the horizontal reclined position. The baseline SBP, MAP, DBP, and HR were recorded before the tilt. The tilt study was performed for 10 min with a 70 degree HUT. Changes in the HR, BP, and symptoms during the tilt were recorded. Finapress was used to record BP continuously from the fingertip. The systolic fall and HR increment at 30 s and at 1, 3, 5, 8, and 10 min were documented. After the HUT, the table was again made horizontal and we again recorded the SBP, MAP, DBP, and HR for 10 min
4. QSART – The QSART assessed postganglionic sudomotor nerve fibers and sweat glands in localized areas of the skin. A multicompartmental sweat cell was used to measure the sweat production. The cell contained an inner compartment – that was filled with 10% acetylcholine chloride dissolved in distilled water. There was also the outer compartment – that took up the humidity from the axon-induced sweat production. The volume of sweat output was calculated automatically by area under the curve method. There were four sites on the extremities:
   i. Forearm – midway along the inner forearm.
   ii. Proximal leg – 3 cm below the head of the tibia over the deep peroneal nerve.
   iii. Distal leg – midway between the tibia head and the lateral malleolus (ankle bone).
   iv. Foot – half distance down the third metatarsal from the toes to the tarsal joint.

   (Reference electrodes were placed at a distance no more than 10 cm from recording site)
5. TST: The lower limbs, upper limbs, and feet of the subjects were dusted with iodine solution (2% iodine powder in 96% ethyl alcohol); then, a paste which was a mixture of 50% starch in castor oil was applied on the skin surface. The subject remained in the TST room that was used to heat the body core temperature, with a heater. The heating time was approximately 45–60 min. Digital photographs were taken after 45 min of heating time to document areas of sweating. After the testing data was expressed as TST%, which was the measured area of sweat (Total area of paste applied – Area of anhidrosis) divided by the total area of paste applied, multiplied by 100.[10] The pixels of total area and the area of paste applied were calculated using Photoshop software with histogram. The sweat area was identified by adding each subject's skin tone to the software.

The manuscript was prepared according to STROBE guidelines [Supplementary Material 1].

Sample size

The sample size was calculated based on our pilot study and previous Indian study[11] by taking mean HR difference values in the age groups 20–30 years, 31–40 years, 41–50 years, 51–60 years, 61–70 years, >=71 years, and allowable error ± 10; the sample size calculated was 254.

Statistical analysis

Normative percentiles were calculated at 2.5 and 97.5% according to age and gender. Kolmogorov–Simonov test was used to check the normality of the data. Correlation of age and BMI with the autonomic parameters was obtained using Pearson correlation or Spearman rank correlation depending upon distribution of the data. Independent *t*-test or Mann–Whitney *U* test was used to obtain association of autonomic parameters with gender. Association of autonomic parameters with different age groups was obtained using one-way ANOVA or Kruskal–Wallis. Linear regression analysis was used to find predictors for autonomic parameters using age, gender, and nonlinear interactions between age and gender when both were found to be significant in the model. The significance level was set at $P < 0.05$. All statistical analysis was performed using SPSS, version 26.0. The photographs for sweat area in TST were analyzed using Photoshop CC software (PHSP, ALL, MLP, DRI01, MUA, 001, N/A, 1 MO, DSP).

Results

The mean (SD) age of the subjects was 43 (16.0) years (range 20–84 years) and 129 (50.8%) were males. The mean (SD) BMI, SBP, DBP, and random blood sugar (RBS) were as follows: BMI 23.4 (2.1) (range 18.1–26.9 kg/m^2), SBP 123 (9.53) (range 100–146 mm Hg), DBP 77 (6.57) mm Hg (range 65–98 mm Hg), and RBS 98 (14.53) mg/dL (range 71–140 mg/dL). The AFT parameters were analyzed for 249 subjects after removing the outliers.

HRDB

The average HR difference and E: I ratio in participants were 21.1 (9.09) (range 3.5–47.0 beats per minute) and 1.35 (0.18) (range 1.05–1.93), respectively. The normative data values for HR difference and E: I ratio were calculated at 2.5th and 97.5th percentiles according to age and gender [Table 1]. HR_{DB} difference showed an inverse relationship with age $r = -0.623$, $P < 0.0001$ [Figure 1], but no relation was observed with gender and BMI.

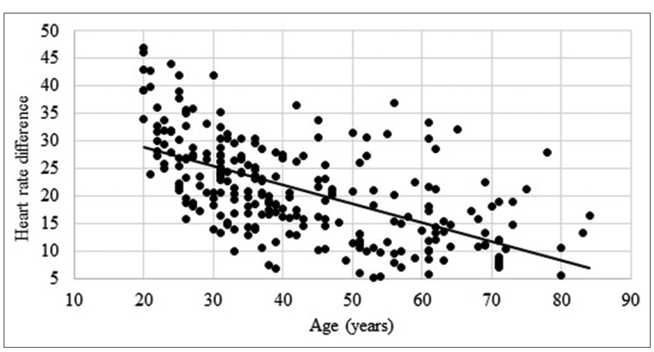

Figure 1: Correlation of age with average difference in heart rate responses to deep breathing

VM

The mean (SD) VR for all the participants was 1.71 (0.30) (range 1.11–2.64). The normative data values for VR was calculated at 2.5th and 97.5th percentiles according to age and gender [Table 2]. VR had an inverse relationship with age $r = -0.575$, $P < 0.0001$ [Figure 2] and was significantly higher in males 1.79 (0.31) compared to females 1.63 (0.27), $P < 0.0001$.

In different phases of VM, the SBP amplitude in early phase 2 showed an inverse relationship with age $r = -0.309$, $P < 0.0001$ and a slight positive correlation was seen with phase 4, $r = 0.236$, $P < 0.0001$ [Figure 3(a and b)]

The normative data values for BP recovery time PRT 100 and PRT 50 were calculated at 2.5th and 97.5th percentiles according to age and gender [Supplementary Material 2]. The BP recovery time (seconds) had a small positive correlation with age at complete recovery and 50% recovery from phase 3 to baseline; $r = 0.244$, $P =< 0.0001$ and $r = 0.264$, $P =< 0.0001$, respectively. VM showed no correlation with BMI.

HUT study

In HUT, a slight SBP fall was found in male compared to female at 30 s and 3 min of tilt up ($P = 0.018$ and $P = 0.002$, respectively), but the difference was nonsignificant for other intervals of time [Supplementary Material 2]. The HR increment from pretilt to tilt up showed no significant correlation with gender. There was no relation found between HUT and age. The normative data values for SBP fall and HR increment were calculated at 2.5th and 97.5th percentiles according to age and gender [Table 3].

QSART

The mean (SD) sweat volume for: forearm, proximal leg, distal leg, and foot was 0.596 (0.326) μL (0.116–2.721 μL), 0.596 (0.318) μL (range 0.106–2.612 μL), 0.609 (0.281) μL (0.125–2.141 μL), and 0.509 (0.232) μL (range 0.127-1.677 μL), respectively. The normative data values for all the four sites were calculated at 2.5th and 97.5th percentiles according to age and gender [Table 4]. The association between sweat volume and gender showed males had significantly more sweat volume compared to females for all the sites [Supplementary Material 2]. In association with age, a decreasing trend was found in sweat volume which was not significant for proximal leg and forearm, but a significant difference was found for

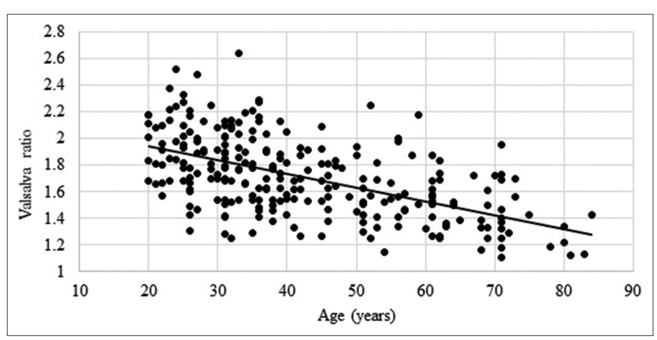

Figure 2: Correlation of age with Valsalva ratio in Valsalva maneuver

Table 1: Normative data values for heart rate difference and E:I ratio distributed according to age and gender in heart rate responses to deep breathing

Heart rate difference (beats per minute)							
Male	**Mean (SD)**	**95% Normative values (2.5th percentile to 97.5th percentile)**		**Female**	**Mean (SD)**	**95% Normative values (2.5th percentile to 97.5th percentile)**	
20-30	29.43 (7.92)	17.08	44.68	20-30	29.47 (8.58)	16.86	43.83
31-40	22.70 (5.90)	13.40	31.30	31-40	20.62 (6.09)	9.88	30.51
41-50	22.06 (8.21)	11.78	35.49	41-50	17.31 (4.71)	9.05	23.13
51-60	14.10 (7.94)	7.74	29.73	51-60	16.25 (8.83)	8.08	34.91
61-70	15.03 (6.32)	5.58	26.71	61-70	14.50 (6.77)	6.68	29.27
≥71	12.46 (7.97)	3.61	26.42	≥ 71	11.23 94.34)	5.84	18.34
E:I ratio							
Male	**Mean (SD)**	**95% Normative values (2.5th percentile to 97.5th percentile)**		**Female**	**Mean (SD)**	**95% Normative values (2.5th percentile to 97.5th percentile)**	
20-30	1.51 (0.18)	1.23	1.88	20-30	1.49 (0.19)	1.25	1.87
31-40	1.39 (0.12)	1.18	1.62	31-40	1.33 (0.12)	1.12	1.55
41-50	1.36 (0.16)	1.16	1.60	41-50	1.28 (0.08)	1.14	1.39
51-60	1.27 (0.14)	1.13	1.53	51-60	1.25 (0.16)	1.10	1.59
61-70	1.25 (0.11)	1.08	1.46	61-70	1.23 (0.14)	1.10	1.56
≥71	1.19 (0.14)	1.05	1.44	≥ 71	1.17 (0.09)	1.07	1.35

Table 2: Normative data values for Valsalva ratio distributed according to age and gender in Valsalva maneuver

VR	Mean (SD)	95% Normative values (2.5th percentile to 97.5th percentile)	
Male			
20-30	2.01 (0.22)	1.68	2.49
31-40	1.88 (0.25)	1.50	2.29
41-50	1.74 (0.14)	1.49	1.94
51-60	1.56 (0.17)	1.32	1.84
61-70	1.53 (0.21)	1.18	1.80
≥71	1.39 (0.29)	1.11	1.78
Female			
20-30	1.79 (0.25)	1.40	2.24
31-40	1.68 (0.26)	1.28	2.14
41-50	1.61 (0.24)	1.27	2.03
51-60	1.55 (0.23)	1.27	1.99
61-70	1.45 (0.18)	1.26	1.80
≥71	1.42 (0.18)	1.23	1.70

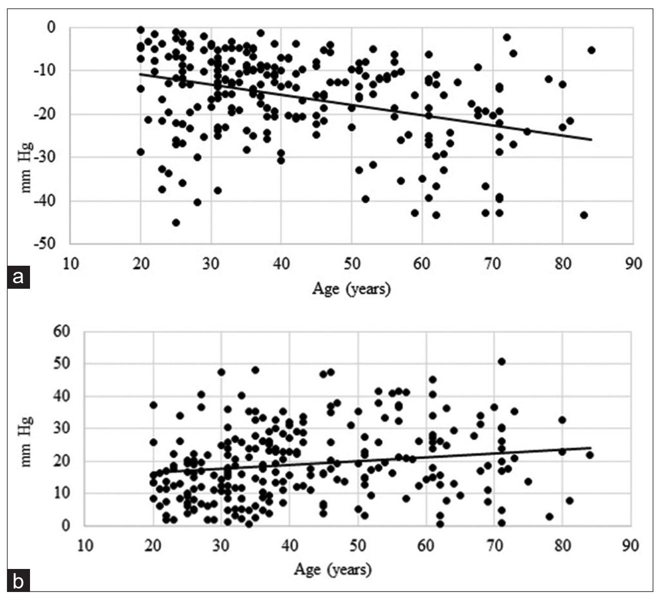

Figure 3: (a) Correlation of age with systolic blood pressure (SBP) in early phase 2 amplitude during Valsalva maneuver (b) Correlation of age with systolic blood pressure (SBP) in phase 4 amplitude during Valsalva maneuver

distal leg and foot [Supplement Material 2]. There was no correlation with BMI.

TST

The mean age (SD) was 40 (15.4) years (range 20–84). The normative value range (2.5–97.5 percentile) of sweat area for posterior lower limb (LL), anterior LL, posterior upper limb (UL), anterior UL, and dorsal and plantar surfaces of feet was calculated for all the subjects [Table 5]. The area of sweat was found to be significantly larger in males than females for lower limb, upper limb, and feet [Supplementary Material 2]. The sweat area did not correlate with age and BMI.

The multiple linear regression model was obtained to measure the effect of AFT parameters with age and gender of participants [Supplementary Material 2]. In HRDB, HR difference and E:I ratio showed a significant effect with age but not gender. In VM, VR showed a significant relation with age, gender, and age by gender interaction, the effect of VR was inverse with age, and females had negative effect showing a lower value for VR compared to male. PRT 100 and PRT 50 both had a significant effect with age, but no effect was noticed with gender in a model with interaction factor of age by gender. In HUT, HR increment had a negative relationship with age at 1, 3, and 5 min of tilt up. SBP showed an effect with

Table 3: Normative data values for systolic BP fall and HR increment at different intervals of time for all the age groups in HUT

Systolic Blood Pressure fall (mmHg)							
Male	**Median (IQR)**	**95% Normative values (2.5th percentile to 97.5th percentile)**		**Female**	**Median (IQR)**	**95% Normative values (2.5th percentile to 97.5th percentile)**	
At 30 s	-1.3 (-6.8-3.1)	-18.2	14.4	At 30 s	0.2 (-4.3-6.0)	-20.1	15.7
At 1 min	-2.3 (-7.2-2.8)	-18.5	17.7	At 1 min	0 (-5.3-4.5)	-17.2	17.0
At 3 min	-2.4 (-6.6-1.6)	-19.2	15.1	At 3 min	0.2 (-4.9-5.6)	-17.8	16.4
At 5 min	-2.0 (-7.1-2.2)	-17.0	17.9	At 5 min	-1.5 (-6.1-5.5)	-19.3	15.9
At 8 min	-2.3 (-5.9-2.3)	-21.4	15.1	At 8 min	-0.8 (-5.6-5.5)	-21.4	19.9
At 10 min	-0.8 (-5.3-4.0)	-25.6	13.2	At 10 mins	0.5 (-4.3-7.3)	-26.2	18.7
Average	-1.8 (-5.1-1.3)	-14.9	12.1	Average	0.3 (-3.4-3.9)	-18.6	12.1
Hear rate increment (beats per minute)							
Male	**Median (IQR)**	**95% Normative values (2.5th percentile to 97.5th percentile)**		**Female**	**Median (IQR)**	**95% Normative values (2.5th percentile to 97.5th percentile)**	
At	0.8 (-3.5-5.1)	-11.4	15.8	At 30 s	2.0 (-1.8-4.5)	-10.1	15.3
At 1 min	3.8 (-0.6-7.9)	-12.4	19.1	At 1 min	2.7 (-1.9-6.4)	-11.2	17.8
At 3 min	6.7 (-0.6-13.1)	-11.1	24.8	At 3 min	3.6 (-1.1-10.3)	-9.8	22.6
At 5 min	6.3 (-0.5-13.1)	-9.7	21.4	At 5 min	5.5 (0.8-11.3)	-7.4	20.9
At 8 min	5.8 (-1.8-13.4)	-11.8	25.0	At 8 min	3.5 (-0.9-10.9)	-6.0	20.0
At 10 min	1.7 (-3.5-9.0)	-10.4	22.9	At 10 min	2.2 (-2.3-8.0)	-10.7	19.1
Average	4.2 (-0.2-9.1)	-8.6	17.0	Average	3.4 (0.4-6.3)	-5.4	17.5

gender at 3 min tilt-up, but no relationship was seen with age. In QSART, sweat volume in proximal leg, distal leg, and foot had a significant inverse relation with age, and the effect of sweat volume in females was negative indicating lower values.

Discussion

We documented the normative data for cardiovascular AFT, QSART, and TST among Indian subjects. In HRDB, we found an inverse linear relationship with age which persisted beyond 60 years of age, which is important to consider when examining patients especially in the older age group. A linear progressive reduction with age in HRDB has been seen in various studies.[9,12,13] Our study also shows that HRDB in subjects over 70 years does not approach zero or level out, in contrast to what was suggested in previous study.[12] The values for HR difference and E: I ratio are similar to what has been previously reported in the younger age group.[6] These values though low among those older than 50 years were similar to another study from India.[11] There was no relation observed with gender and BMI correlating with previous studies.

Correlation of VR with age has been variable according to different studies. Some studies have mentioned that VR has no correlation with age, while other studies have shown an inverse correlation with age.[6,13] We found a clear inverse correlation with age. Previous studies have been varied regarding the effect of gender on VR.[6,14] Our study found the VR to be higher in males 1.79 (0.31) compared to females 1.63 (0.27), $P < 0.0001$. Moreover, the pattern was inversely correlating with age in both males and females unlike previously reported.

VR is more complex and has multiple factors affecting it (blood volume, rest, cardiac sympathetic and peripheral sympathetic tone, and nor-adrenaline response) unlike HRDB, which is mainly influenced by cardiovagal reflex. Our study showed different phases of VM like the SBP amplitude in early phase 2 showed a moderate inverse relationship with age and a slight positive correlation was seen with phase 4. This finding corroborates the fact that age affects different components of Valsalva in different directions.[14] PRT 100 and PRT 50 had a small positive correlation with age, which has not yet been reported.[14,15] There was no correlation with gender or BMI.

In the HUT study, we found that SBP fall and HR increment showed no correlation with age or gender. Previous studies mention a positive correlation with SBP fall and negative correlation with HR increment for age peaking beyond 70 years.[6,14] This may be due to the smaller sample size.

In agreement with other studies, we noted that males had significantly more sweat volume (0.630 ± 0.230) compared to females (0.513 ± 0.132) at all sites.[6,13] This occurs due to smaller evoked sweat volume per sweat gland, rather than reduction in number of sweat glands in females.[16] We found a decrease in sweat volume with age for distal leg and foot, while the proximal leg showed a decreasing trend not reaching statistical significance. However, the forearm showed no change with age, which is concordant with other studies. This can be explained by the fact that longer unmyelinated fibers have been shown to be affected by age preferentially.[6] We found that the range of sweat volume in our population was similar to studies done in India and Taiwan but differed from

Table 4: Normative data values for sweat volume (μL) in fore arm, proximal leg, distal leg, and foot for QSART

Fore arm							
Male	**Mean (SD)**	**95% Normative values (2.5th percentile to 97.5th percentile)**		**Female**	**Mean (SD)**	**95% Normative values (2.5th percentile to 97.5th percentile)**	
20-30	0.777 (0.61)	0.153	2.302	20-30	0.501 (0.18)	0.230	0.900
31-40	0.671 (0.29)	0.313	1.367	31-40	0.531 (0.17)	0.234	0.840
41-50	0.639 (0.34)	0.295	1.403	41-50	0.533 (0.26)	0.196	1.106
51-60	0.691 (0.28)	0.342	1.217	51-60	0.533 (0.12)	0.332	0.722
61-70	0.679 (0.38)	0.383	1.612	61-70	0.495 (0.17)	0.215	0.754
≥ 71	0.509 (0.27)	0.379	1.554	≥ 71	0.434 (0.13)	0.292	0.683
Proximal leg							
Male	**Mean (SD)**	**95% Normative values (2.5th percentile to 97.5th percentile)**		**Female**	**Mean (SD)**	**95% Normative values (2.5th percentile to 97.5th percentile)**	
20-30	0.810 (0.42)	0.135	2.373	20-30	0.522 (0.24)	0.224	1.003
31-40	0.627 (0.19)	0.410	1.502	31-40	0.551 (0.20)	0.243	0.899
41-50	0.618 (0.21)	0.322	0.977	41-50	0.508 (0.29)	0.177	1.161
51-60	0.564 (0.20)	0.342	0.896	51-60	0.563 (0.16)	0.361	0.850
61-70	0.667 (0.28)	0.419	1.264	61-70	0.495 (0.17)	0.226	0.848
≥ 71	0.459 (0.19)	0.393	1.293	≥ 71	0.461 (0.15)	0.337	0.723
Distal leg							
Male	**Mean (SD)**	**95% Normative values (2.5th percentile to 97.5th percentile)**		**Female**	**Mean (SD)**	**95% Normative values (2.5th percentile to 97.5th percentile)**	
20-30	0.806 (0.39)	0.240	1.754	20-30	0.549 (0.17)	0.285	0.940
31-40	0.614 (0.20)	0.318	1.334	31-40	0.556 (0.17)	0.283	0.873
41-50	0.625 (0.18)	0.345	0.864	41-50	0.498 (0.19)	0.197	0.861
51-60	0.591 (0.21)	0.342	0.840	51-60	0.563 (0.12)	0.444	0.810
61-70	0.694 (0.28)	0.354	1.297	61-70	0.504 (0.13)	0.331	0.747
≥ 71	0.467 (0.15)	0.349	1.269	≥ 71	0.452 (0.13)	0.328	0.701
Foot							
Male	**Mean (SD)**	**95% Normative values (2.5th percentile to 97.5th percentile)**		**Female**	**Mean (SD)**	**95% Normative values (2.5th percentile to 97.5th percentile)**	
20-30	0.626 (0.27)	0.182	1.314	20-30	0.466 (0.14)	0.219	0.710
31-40	0.540 (0.21)	0.217	0.860	31-40	0.503 (0.17)	0.264	0.886
41-50	0.546 (0.24)	0.236	1.078	41-50	0.521 (0.31)	0.210	1.246
51-60	0.455 (0.14)	0.284	0.711	51-60	0.464 (0.15)	0.276	0.787
61-70	0.658 (0.39)	0.300	1.604	61-70	0.462 (0.13)	0.280	0.664
≥71	0.328 (0.08)	0.299	1.555	≥ 71	0.374 (0.09)	0.294	0.559

Table 5: Normative data values for sweat area in feet, lower limb, and upper limb for TST

	Mean (SD) (%)	95% Normative values (2.5th percentile to 97.5th percentile)
Feet		
Dorsal	69.93 (9.11)	51.0-87.02
Plantar	65.08 (9.63)	44.18-78.47
Lower limb		
Posterior	69.29 (10.61)	41.85-87.39
Anterior	72.19 (9.79)	48.52-90.67
Upper limb		
Posterior	75.45 (9.07)	50.15-88.75
Anterior	76.03 (10.77)	49.02-91.44

studies on Western population. There is some suggestion of ethnic differences playing a role.[17-19]

We performed TST on 50 subjects and found the area of sweat to be significantly larger in males than females for lower limb, upper limb, and feet. Studies have suggested that females have different threshold for thermoregulation, which may account for this finding.[20] In our study, the sweat area did not correlate with age and BMI.

The strengths of this study are, first all the subjects were screened for diseases and medications affecting the ANS and they were prepared uniformly for the tests which were performed under similar controlled environment by the same person using standardized equipment with software. Second, the sample size was calculated based on a previous study from the country and was further modified to enroll a larger sample using data from a pilot study.[11] Third, we also used a new method to quantify TST.

The limitations are the small study population over the age of 40. This makes it difficult to draw definite conclusions for this age group. Finding subjects fulfilling all exclusion criteria in this age group was a challenge. This challenge is represented across other studies as well.[14,21-23] Furthermore, QSART was studied on one side of the body and TST was performed only on the extremities.

Conclusions

We have derived the normative data for AFTs in India. Our study demonstrates the influence of age on HRDB, VR, and QSART. Gender differences were demonstrated in VR, QSART, and TST.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request

Ethics approval

The approval for the study was obtained from institutional review board Christian Medical College and Hospital, Ludhiana.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient (s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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Supplement Material 1

STROBE Statement-Checklist of items that should be included in reports of *cohort studies*			
	Item no	**Recommendation**	**Page no**
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	2
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	3-5
		(b) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	3-5
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	5
		(b) Describe any methods used to examine subgroups and interactions	NA
		(c) Explain how missing data were addressed	NA
		(d) If applicable, explain how loss to follow-up was addressed	NA
		(e) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6-8
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	Report numbers of outcome events or summary measures over time	NA
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(b) Report category boundaries when continuous variables were categorized	NA
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done-eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	8-10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9-10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10

Supplement Material 2

Table 1: Normative data values for Pressure recovery time PRT 100 and PRT 50 in Valsalva Maneuver

PRT100							
Male	Mean (SD)	95% Normative values (2.5th percentile to 97.5th percentile)		Female	Mean (SD)	95% Normative values (2.5th percentile to 97.5th percentile)	
20-30	2.53 (0.82)	1.44	4.65	20-30	2.11 (0.51)	1.28	3.04
31-40	2.57 (0.90)	1.62	4.69	31-40	2.48 (0.94)	1.50	4.56
41-50	2.36 (0.63)	1.59	3.62	41-50	2.47 (0.63)	1.44	5.28
51-60	2.84 (0.74)	1.77	4.18	51-60	2.79 (1.08)	1.38	5.00
61-70	3.71 (1.56)	2.01	6.86	61-70	2.98 (1.09)	1.49	4.64
≥71	5.99 (3.41)	1.29	10.67	≥71	3.16 (1.95)	1.43	7.05
PRT50							
Male	Mean (SD)	95% Normative values (2.5th percentile to 97.5th percentile)		Female	Mean (SD)	95% Normative values (2.5th percentile to 97.5th percentile)	
20-30	1.16 (0.38)	0.59	1.95	20-30	0.97 (0.22)	0.70	1.50
31-40	1.22 (0.40)	0.72	2.25	31-40	1.18 (0.44)	0.64	2.03
41-50	1.11 (0.35)	0.69	1.79	41-50	1.21 (0.61)	0.74	2.58
51-60	1.33 (0.33)	0.89	1.93	51-60	1.25 (0.46)	0.70	2.23
61-70	1.81 (0.91)	0.89	3.90	61-70	1.35 (0.47)	0.68	2.07
≥71	2.71 (1.55)	0.54	4.88	≥71	1.52 (1.05)	0.75	3.71

Table 2: Association of gender with systolic blood pressure fall (mmHg) from baseline to tilt-up at different intervals of time

Systolic blood pressure fall	Gender (Median (IQR))		
Pre-tilt to Tilt-up	Male	Female	*P*
Average	-1.8 (-5.0-1.3)	0.3 (-3.4-3.9)	0.002
At 30 sec	-1.2 (-6.8-2.6)	0.2 (-4.3-5.9)	0.018
At 1 min	-2.3 (-6.9-2.8)	0 (-5.3-4.5)	0.056
At 3 mins	-2.5 (-6.5-1.5)	0.2 (-4.9-5.6)	0.002
At 5 mins	-2.0 (-7.0-2.1)	-1.5 (-6.1-5.5)	0.142
At 8 mins	-2.2 (-5.5-2.5)	-0.8 (-5.6-5.5)	0.087
At 10 mins	-0.8 (-5.3-3.6)	0.5 (-4.3-7.3)	0.083

Table 3: Sweat volume (μL) in participants from Quantitative Sudomotor Axon Reflex Test (QSART) association with gender

	Gender (Mean (SD))		
	Male	Female	*P*
Front arm	0.678 (0.407)	0.512 (0.178)	<0.0001
Proximal Leg	0.663 (0.384)	0.526 (0.211)	<0.0001
Distal Leg	0.682 (0.346)	0.533 (0.162)	<0.0001
Foot	0.541 (0.271)	0.477 (0.179)	0.026

Table 4: Sweat volume (μL) in participants from Quantitative Sudomotor Axon Reflex Test (QSART) association with age

Age-groups (years)	Mean (SD)			
	Front arm	Proximal Leg	Distal Leg	Foot
20-30	0.644 (0.48)	0.671 (0.48)	0.708 (0.39)	0.536 (0.26)
31-40	0.602 (0.25)	0.605 (0.24)	0.598 (0.22)	0.522 (0.19)
41-50	0.586 (0.30)	0.563 (0.26)	0.561 (0.19)	0.537 (0.28)
51-60	0.574 (0.20)	0.576 (0.24)	0.584 (0.30)	0.449 (0.14)
61-70	0.599 (0.31)	0.579 (0.23)	0.606 (0.24)	0.555 (0.30)
≥71	0.472 (0.21)	0.435 (0.16)	0.459 (0.13)	0.351 (0.09)
P	0.165	0.055	0.024	<0.001

Table 5: Association Sweat area (%) from Thermoregulatory sweat test (TST) with gender

Sweat area (%)	Gender Mean (SD) (%)		
	Male	Female	*P*
Feet			
Dorsal	74.88 (7.62)	64.98 (7.79)	<0.0001
Plantar	71.29 (5.72)	58.86 (8.74)	<0.0001
Lower limb			
Posterior	74.95 (7.97)	63.63 (9.97)	<0.0001
Anterior	76.30 (9.98)	68.08 (7.81)	0.002
Upper limb			
Posterior	79.51 (7.08)	71.39 (9.15)	0.001
Anterior	81.23 (8.47)	70.83 (10.41)	<0.0001

Table 6: Multiple linear regression model measuring the effect of AFT parameters with predictors

	Age	Gender	Age × Gender
HRDB			
Hear rate difference	-0.342*	-1.215	
E: I ratio	-0.006*	-0.033	
VM			
Valsalva ratio	-0.013*	-0.348*	0.005*
PRT100	0.048*	0.737	-0.028*
PRT50	0.022*	0.352	-0.013*
HUT			
SBP 1 min	-0.056	1.600	
HR 1 min	-0.093*	-1.217	
SBP 3 mins	-0.038	3.116*	
HR 3 mins	-0.121*	-1.555	
SBP 5 mins	-0.033	1.546	
HR 5 mins	-0.153*	-0.459	
QSART (Sweat volume)			
Front arm	-0.003*	-0.239*	0.002
Proximal leg	-0.005*	-0.291*	0.004
Distal leg	-0.004*	-0.216*	0.002
Foot	-0.003*	-0.137	0.002

*$P<0.005$