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Characterization of pharyngeal peristaltic pressure variability during volitional swallowing in healthy individuals

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Abstract

Background: Recent technological advances incorporated in high resolution manometry have justifiably heightened interest in manometric evaluation of the pharynx. Despite this interest, from both physiologic and clinical perspective there remain a number of unanswered questions regarding the magnitude of variability of pharyngeal pressure phenomena. Therefore, the aim of the present study was to characterize in healthy individuals the inter-subject and recording-site specific variability of pharyngeal peristaltic pressure phenomena.

Methods: We studied 32 healthy subjects (age: 21–83 years, 20 under 35 years) during dry, 5 and 10 mL water swallows $\times 3$. Pharyngeal peristaltic pressures were recorded using a high resolution (HR) manometric system and a catheter assembly with 36 circumferential sensors spaced at 1 cm intervals positioned trans-nasally to traverse the pharynx, UES and proximal esophagus.

Key Results: Both site-specific pressure data and the Pharyngeal contractile integral (PhCI) showed wide dispersion ranging between values under 50 mm Hg to over 300 mm Hg and 100–600 mm Hg/cm/s, respectively. There was also wide range of dispersion of data for both the standard deviations and the coefficient of variation for all sites ($P = .001$). The coefficient of variation for PhCI ranged between 0.02 and 0.25 representing data dispersion of 2–25 percent of the mean among subjects ($P = .001$). Position, age and volume of swallowed fluid did not influence the magnitude of variability.

Conclusions: Deglutitive Pharyngeal peristalsis generates pressures with significant degree of site-related and inter-subject variability. This variability is not influenced by age, position and volume of swallowed fluid.

Keywords

deglutition; dysphagia; peristalsis; pharyngeal manometry; swallowing; variability

1 | INTRODUCTION

Until recently, evaluation of deglutitive pharyngeal pressure phenomena outside the research protocols has not been possible due to unavailability of recording devices that could reliably

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CONFLICT OF INTEREST

None.

record the radially and axially asymmetric rapid pressure transients of the entire length of the pharynx. Earlier studies using solid state unidirectional as well as circumferential pressure sensors evaluated the distal pharynx and have provided valuable physiologic data on the effect of bolus volume, osmolality, and texture as well as the effect of position and age on the hypopharyngeal peristaltic pressure amplitude, duration, velocity and intra-bolus hypopharyngeal pressure.¹⁻⁵ However, due to cumbersome technical and analytical issues, this approach could not have been used routinely in clinical setting.

Recent technological advances which have overcome a number of shortcomings of the older recording modalities by providing narrowly spaced sensors with circumferential recording ability, accept rise rate, ease of catheter positioning, ability to record from the entire pharyngeal lumen and sophisticated data displaying have justifiably heightened interest in manometric evaluation of the pharynx. This interest has spurred a number of studies using High Resolution Manometry (HRM) which incorporates these new technological advances.⁶⁻¹⁴ Despite the availability of large amount of intra-pharyngeal pressure data by these recent studies, there are unanswered questions regarding the magnitude of variability of the pressure data within the length of contractile pharynx and among individuals. Addressing these questions are important from both physiologic and clinical perspective. The aims of the present study therefore were to characterize in healthy individuals the intra-and inter-subject as well as the recording-site specific variability of pharyngeal peristaltic pressure phenomena.

2 | METHODS

We studied 32 healthy subjects (age: 34 ± 16 years standard deviation(SD), range 21–83 years, 12 female, 20 younger than 35 years) by recording pressure in the pharynx, upper esophageal sphincter (UES) and proximal esophagus during sequences of dry, 5 and 10 mL room temperature water swallows. Studies were approved by the internal review board of the Human Research Protection Program at the Medical College of Wisconsin, Milwaukee, Wisconsin. All participants gave written informed consent prior to their studies.

2.1 | Experimental procedures

Pharyngeal peristaltic pressures were recorded using high resolution manometry system. A HRM catheter assembly with 36 circumferential sensors spaced at 1 cm intervals (outer diameter, 4.2 mm) was positioned trans-nasally to traverse the pharynx, UES and proximal esophagus after application of 2% viscous lidocaine. Transducers on the catheter were calibrated at 0 and 300 mm Hg using externally applied pressure. The response characteristics of each sensing element were such that they could record pressure transients in excess of 6000 mm Hg/s and were accurate to within 1 mm Hg of atmospheric pressure. The data acquisition frequency was 50 Hz for each sensor. The HRM probe and computerized recording and analysis system (ManoScan and ManoView Systems, Given Imaging, Inc., GA, USA) stores pressure data from all 36 pressure sensors on the probe, displays the manometric information in topographic or line graph formats as well as provides postacquisition analytic tools for parameterization of temporal and spatial pressure data.

2.2 | Study protocol

Study subjects fasted for at least 6 hours before their studies. Studies were performed with subjects upright and sitting with their head in neutral position. After an initial 10 minute adaptation period to mitigate pharyngeal pressure transients after introduction of the HRM catheter, each subject completed three repetitions of each of the following swallows with 20 seconds intervals: dry swallow and 5 and 10 mL room temperature water swallows. The water boluses were slowly injected into the oral cavity by a syringe and the subjects were then cued to swallow the water in a single swallow. Swallow conditions were repeated in supine position in 16 subjects. The order of swallows was randomized for each subject.

2.3 | Measured parameters

Several manometric parameters were measured and analyzed for each swallow. Peak deglutitive peristaltic wave pressures were measured at positions 2, 3, 4, 5, 6, 7 and 8 cm above the upper margin of the mano-metrically determined upper esophageal sphincter (UES). Additionally, a parameter derived from the ManoView analysis software, that is, pharyngeal contractile integral was measured. The pharyngeal contractile integral (PhCI) was calculated using the “SmartMouse” feature of the ManoView software. The contractile integral technique has been utilized in the distal esophagus as metric of “contractile vigor”¹⁵ by multiplying the mean pressure amplitude times the contraction duration times the length of the region of interest. In the ManoView software topographic display using the computer’s mouse, the contractile integral is calculated by scrolling out an area in the topographic display delineating a space-time box and logging the displayed contractile integral value. For the purposes of our analysis, the PhCI was characterized by circumscribing a space-time box in the topographic ManoView display to surround the pharyngeal deglutitive pressure recording with the upper margin of the box at the most proximal probe sensor at a time just prior to start of deglutition and the distal margin of the box at the predetermined upper margin of the UES high pressure zone at the time of return of the high pressure zone to its resting manometric profile (Figure 1). All pressure measurements were referenced to atmospheric pressure.

2.4 | Statistical analyses

Within and between subject effects were tested using analysis of variance (ANOVA) as well as multiple linear regression for parametric data sets and Friedman’s Test for non-parametric data sets. The Shapiro-Wilk test was used to determine whether a set of sample data likely came from a normally distributed population.

In addition to testing the difference in mean or median of the sampled data, the dispersion of the collected data was also tested to determine potential differences in the variability of the site-wise and integrated pressure data. The metrics for dispersion were the SD and the coefficient of variation (CV) defined as the ratio of the standard deviation divided by the mean therefore providing a dimensionless metric parameterizing the variability relative to the mean.

All data are expressed as mean $\pm$ SD. Comparison of standard deviation and coefficient of variation were performed using Bartlett’s Test of Variance and Conover’s Squared Rank Test

of Variance. Two-tailed $P < .05$ were considered statistically significant. Effect of age, swallow volumes and subject position were compared using ANOVA.

3 | RESULTS

3.1 | Variability of peak peristaltic pressures

All subjects tolerated the experiment well without any complaint or complications related to the study procedure.

To identify the location of the intra-pharyngeal recording sites relative to the UES, the proximal margin of the UES high pressure zone (UESHPZ) was defined by the most oral 20 mm Hg isobar shown on the manometric contour plot and all the pharyngeal recording sites were referenced to this spatial landmark.

The effect of manometric recording location on peak peristaltic wave pressure for dry, five and ten mL water swallows are shown in Figure 2. As seen, although the mean peak pharyngeal peristaltic pressures were comparable between sites for dry, 5 mL water swallows and 10 mL water swallows there was a wide range of average pressures among subjects for each site. These average pressures ranged from values under 50 mm Hg to values over 300 mm Hg among studied subjects. Analysis of variance comparing the pressure values among sites, however, did not show any significant differences for any of the tested swallow volumes ($P = .13$, $P = .21$ and $P = .43$, respectively).

Observation of the scattergram of mean pressure values for all subjects clearly shows that pharyngeal pressure phenomena for all tested volumes exhibit a nadir at location four cm proximal to the UES and the pressure increases in sites proximal and distal to this location. However, this finding does not always represent all subjects. Each individual may exhibit a different nadir pressure site within the contractile pharynx. This variation could have potentially masked statistically significant site related pressure differences. Another factor contributing to lack of statistically significant difference among sites could be the large degree of inter-subject variability as observed in Figure 2.

3.2 | Intrasubject swallow to swallow variability

Within subject variability of peak peristaltic amplitude at each recording site above the upper margin of the UES high pressure zone was tested using nested hierarchical repeated measures ANOVA with replication to assess the degree of variability within the three observations from each subject across all tested boluses. This analysis did not show any statistically significant difference in intra-subject variability for each tested bolus for all the pressure recording sites 2 to 8 cm above the UES ($P = .97$, $.95$, $.98$, $.99$, $.99$, $.99$ and $.99$, respectively).

3.3 | Variability of coefficient of variation and standard deviation in peak peristaltic pressures

Figures 3 and 4 show the coefficient of variation (CV) and standard deviation (SD) in peak peristaltic amplitude for various recording sites during dry, five and ten ml room temperature water swallows. As seen, similar to the peak pressure data shown in Figure 2, there is a wide

range in the magnitude of dispersion of data for both the standard deviations and the coefficient of variation for all sites. For coefficient of variation this ranges between 0.01 and 0.4 across the recording sites. Despite this wide dispersion for each site, however, analysis of variance showed significant differences in CV among sites in terms of the magnitude of peak peristaltic amplitude variability ($P=.001-.024$ for various swallowed volumes).

These metrics for determining the dispersion of the data showed between-site differences that reveal greater variability very near (2 and 3 cm above the upper margin of the UESHPZ) and far away (7 and 8 cm above the UESHPZ) from the UES but lesser variability elsewhere ($P=.001-.024$ and $P=.001$, respectively, Conover's Test).

3.4 | Variability of pharyngeal contractile integral (PhCI)

Figure 5 shows the pharyngeal contractile integral for dry, 5 and 10 mL water swallow and their related standard deviation and coefficient of variation. As seen in Figure 5 panel A there is a wide degree of variation among subjects wherein PhCI ranges between low values of under 100 mm Hg/cm/s to as high as 650 mm Hg/cm/s. Analysis of variance comparing the effect of volume of swallowed water did not show any significant difference between PhCI's generated during dry, 5 and 10 mL water swallows. As seen in Figure 5 panel B and C, similar to data for site specific pressure values, the standard deviation and coefficient of variation show a wide range of dispersion among studied subjects. For coefficient of variation, these values range between 0.02 and 0.25 representing data dispersion of 2–25 percent of the mean among subjects as further demonstrated in the magnitudes of standard deviation seen in Figure 5 panel B.

In addition, intra-subject variability for PhCI was tested using a nested hierarchical repeated measures ANOVA with replication across all subjects and all volumes. There was no significant intra-subject variability for the three swallow repetitions in each subject ($P=.99$) for any swallow type.

3.5 | Effect of position on variability of pharyngeal pressure phenomena

As stated in the methods section, of the 32 volunteers studied in the upright position, 16 were also studied in the supine position in the same session. To determine whether supine position affects the variability of the pharyngeal peristaltic pressure waves, we compared the above reported parameters between upright and supine position. Comparative data for PhCI is shown in Table 1. For 16 subjects with both upright and supine swallowing data, PhCI metrics were compared using paired t tests. As seen in the table, there was no significant difference in mean PhCI, coefficient of variation or standard deviation for any of the tested swallow volumes when comparing upright to supine data. Similarly, data regarding the site related and inter-subject related variabilities did not reveal any statistically significant differences between upright and supine positions (data not shown).

3.6 | Potential effect of wide range of volunteers age on variability of pharyngeal pressure phenomena

As stated in the methods section, the age of our 32 volunteers ranged between 21 and 83 years. To test whether this wide age range could have contributed to the observed variability

of pharyngeal pressure phenomena we compared the measures of variability between volunteers age 20–35 years (n=20) and the entire group. Tables 2, 3 and 4 show the data for mean peak peristaltic amplitude, standard deviation and coefficient of variation of pressure recording sites and PhCI for the 20 young (age<35 years) compared to all tested subjects. As seen the values for measured parameters are quite similar between groups and analysis of variance failed to reveal significant differences ($P>.05$). Also similar were all other studied measures related to site and position (Table 2) ($P>.05$).

4 | DISCUSSION

In this study, we characterized the variability of pharyngeal peristaltic pressure peak amplitude within the contractile pharynx as well as between healthy individuals during dry, 5 and 10 mL water swallow. We also determined the lack of effect on the magnitude of the observed variability of position and volume of swallowed fluid as well as age heterogeneity. Study findings also indicate that, in each healthy individual, pharyngeal pressure phenomena often includes a nadir zone and two peaks on each side of the nadir. Findings indicate that the location of the nadir zone within the pharynx during contraction differs among individuals. This phenomenon has been observed in various settings in prior studies⁸ and has been referred to as pharyngeal pressure Tilde (~). What the current study adds to these prior findings is the focus on the magnitude and characterization of the variability of this nadir and its associated neighboring peaks and the fact that the observed peak-nadir-peak pharyngeal pressure Tilde has different spatial characteristics unique to each healthy individual. “The reason for this pattern is not completely understood but potential candidates include (i) influence of rigid anatomical structures such as tip of epiglottis or larynx partially shielding the pressure sensor from the compression effect of the muscular contraction, (ii) simply the existence of a normal comparatively low contractility of mid pharynx compared to more proximal or distal regions or (iii) contribution of structures such as velum and soft palate in generating higher pressure proximally and the base of the tongue adding to pressure distally contributing to the appearance of the observed peak pressure trough within the pharyngeal pressure signature”.

Several prior studies using high resolution manometry technique have quantified the pharyngeal deglutitive pressure waves in healthy individuals.^{7,11,14} Findings of the present study are in general concordance with these earlier reports regarding the magnitude of pharyngeal peristaltic pressure waves.

The present study also indicates significant inter-subject variability of the deglutitive peristaltic pressure wave amplitude at each of the recording sites within the contractile pharynx as well as their associated pharyngeal contractile integral (PhCI). PhCI is a function of the pharyngeal pressure amplitude and duration as well as the length of the contractile zone, and provides a single metric of the overall pharyngeal contraction. However, despite this advantage, PhCI suffers a significant shortcoming, in that it masks the site specific pressure parameters. From both physiologic as well as dysphagia management perspectives, this lack of site specific clarity can present a significant impediment in determining the effect of potential rehabilitative interventions aimed at different muscle groups for improving the swallow function.

Contrary to the esophagus, a number of muscle groups are involved in pharyngeal motor function^{13,16} and the pharyngeal deglutitive peristaltic pressure wave is generated by different muscle groups. For example, the generation of the most proximal part of the pharyngeal peristaltic pressure zone is mostly attributable to the contraction of the proximal pharyngeal constrictor and the velum, the middle part of pharyngeal contraction is attributable to the contraction of middle constrictor and tongue base while the pressures generated in the most distal part are attributed to the contraction of the distal pharyngeal constrictor and retraction/thrust of the tongue base. All these are complimented by the contraction of pharyngeal longitudinal muscles contributing to pharyngeal shortening along with contraction of extrapharyngeal suprahyoid muscles.^{13,16} Each of these muscle groups may be uniquely amenable to and demonstrate a unique response to a given intervention as has been shown in the case of velopharyngeal muscle group exhibiting increased pressure with swallowing of larger water volumes,⁸ a response not reported for the rest of the pharynx.

Future interventional studies aimed at improving pharyngeal motor function which may need to use manometric evaluation of the pharynx for determining outcomes, likely will benefit from site specific pharyngeal pressure recording for establishing impact on the muscle groups presumably affected by the intervention with the understanding that, this effect may be masked by using the pharyngeal contractile integral.

Study findings also clearly show that, similar to site-specific variability, PhCI and its measures of variations, although not affected by position and the volume of swallowed fluid, exhibit significant variation among healthy individuals. This can range between 100 and 600 mm Hg/cm/s and pose a significant limitation to the interpretation of pharyngeal manometric data in disordered conditions when abnormalities are not in the extreme and obvious. It is noteworthy, that the potential contribution of pharyngeal manometry to diagnosis and management of swallowing disorders is likely in cases that diagnosis is not obvious and requires clarification using manometry. The use of manometry in extreme cases with an obvious defect will probably be very limited from a diagnostic perspective and more helpful in follow up of the effect of intervention”.

While the observed significant site-, PhCI- and subject-wise variability of pharyngeal contraction can potentially be overcome by paired comparison studies with each participant being his or her own control, it poses significant issue to establishing a range for normal values for pharyngeal peristalsis due to wide range of inter-subject dispersion of both mean peak pressure and the associated coefficient of variation. This limitation needs to be considered and overcome for potential future use of pharyngeal manometry in clinical practice.

It is noteworthy to consider the fact that one of the weaknesses of the present study is related to the small number of swallow repetitions. While the number of subjects in our study (n=32) offers a robust and adequately powered sample size for testing group-wise variability effects between bolus volumes and study conditions, analysis of within-subject variability is tenuous because of the small number of swallows. In the present study design, three repetitions were performed for each volume in each subject. This meager sample size may

not be large enough to fairly parameterize questions of intra-subject variability. Consequently, this might have contributed to the observed overall variability. Future studies designed with more abundant within-subject sampling are warranted to more comprehensively address questions regarding intra-subject pressure metric variation. Another issue that can potentially influence the manometric recordings of the pharynx includes some anthropometric features such as subject height or more precisely the length of the contractile pharynx. Although we did not stratify patients by their anthropometric features, published reports have noted no significant relationship between pharyngeal length and other anthropometric measures.^{17–19}

Although we did not do a formal comparison between young and elderly individuals in terms of the influence of aging on the observed variability of pharyngeal peristaltic pressure amplitude due to inadequate number of those over the age of 70. Since the age of our 32 volunteers ranged between 21 and 83, we raised the question whether this wide age range could have contributed to the observed variability of pharyngeal pressure phenomena. We hypothesized that if that is the case the observed significant variability should not be seen or become significantly reduced among a more homogenous age group such as those between 21 and 35. To test this statistically we determined the variability among the young homogenous age group (21–35, n=20) and the total heterogeneous group (20–83 years). The comparison showed that the variability did not diminish among the homogenous young group therefore we made the statement that age heterogeneity was not causing the observed variability. Similarly, comparison of the variability measures between positions indicates that the observed variability is not influenced by a particular position. In addition, for the tested volumes of the fluid, the amplitude of the pharyngeal peristaltic pressure wave was not influenced by position either. Similar to other conditions stated above, the absence of position effect is apparent in both site-specific pressures as well as the PhCI. This finding has clinical implications and supports the notion that pharyngeal motility studies can be reliably performed in upright position yielding measurements similar to that of supine position therefore simplifying these studies. This finding is in sharp contrast with that of esophageal motility recordings that are significantly influenced by position and traditionally have been performed in non-physiologic swallow positions namely supine posture to elicit maximum optimal esophageal contractile response to swallowed bolus.

In summary, Deglutitive Pharyngeal peristalsis generates pressures with significant degree of site-related and inter-subject variability which is not influenced by age, position and volume of swallowed fluid. This variability needs to be taken into account in defining normative data.

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Key Points

- Despite recent advancements in pressure recording devices and analysis systems such as those provided by High Resolution Manometry, our understanding of pharyngeal pressure phenomena remains incomplete.
- The current study fills a significant gap by providing novel information regarding the magnitude of variability inherent to pharyngeal pressure phenomena.
- Findings of present study not only increases our understanding of pharyngeal motor function, it can contribute to development of manometric approaches to improve diagnosis and inform outcome of intervention in dysphagic patients.

FIGURE 1.
Pharyngeal pressure topography plot with the black line indicating 20 mm Hg isobar (A).
Line tracings of deglutitive pharyngeal pressures noted at various sites proximal to UES
High pressure zone (B)

FIGURE 2. Scatter gram and average of site specific peak peristaltic pressure 2–8 cm proximal to UES High pressure zone

FIGURE 3.

Scatter gram and mean of the coefficient of variation for peak peristaltic pressure at various sites proximal to UES High pressure zone

FIGURE 4.

Scatter gram and mean of the standard deviation for peak peristaltic pressure at various sites proximal to UES High pressure Zone

FIGURE 5.

Scatter gram and average of the pharyngeal contractile integral (PhCI), and its associated standard deviation and coefficient of variation for dry, 5 and 10 ml water swallows

TABLE 1

PhCI comparison in upright and supine positions

	5 mL				10 mL			
	PhCI (mm Hg/cm/s)	CV	SD (mm Hg/cm/s)	PhCI (mm Hg/cm/s)	CV	SD (mm Hg/cm/s)	PhCI (mm Hg/cm/s)	SD (mm Hg/cm/s)
Supine	186±83	0.14±0.14	24±12	200±83	0.12±0.08	29±13	204±101	22±12
Upright	184±71	0.12±0.10	19±11	182±76	0.17±0.15	29±19	194±90	24±23
P value	.87	.52	.50	.08	.26	.25	.30	.80

Upright and supine values are expressed as mean PhCI ± SD. The P value is the chance of making a Type I error when testing the null hypothesis of no difference in the upright and supine PhCI values when comparing within subject mean PhCI, coefficient of variation (CV) and standard deviation (SD) values.

TABLE 2

Mean peak pharyngeal peristaltic amplitude and PhCI for subjects of age<35 years (n=20) years compared to all subjects (n=32)

Mean	Age groups	P2 (mm Hg)	P3 (mm Hg)	P4 (mm Hg)	P5 (mm Hg)	P6 (mm Hg)	P7 (mm Hg)	P8 (mm Hg)	PhCI (mm Hg/cm/s)
Dry	<35 years All	144±56 156±86	142±61 150±65	107±31 115±36	114±53 120±44	113±46 129±46	111±84 127±53	128±45 131±76	229±134 283±144
5 mL	<35 years	139±64	125±49	104±31	111±48	121±45	121±45	106±85	231±119
	All	145±66	144±64	109±29	117±43	132±57	137±57	137±81	280±125
10 mL	<35 years All	134±74 149±74	121±53 142±724	108±44 112±38	111±51 117±45	124±52 135±51	131±46 143±56	107±63 140±90	240±120 288±129

There was no significant difference ($P>0.05$) in any of the average coefficient of variation (CV) values when comparing younger subjects with the larger study cohort. As seen, data from young subjects was not different in magnitude when compared to values across all studied subjects. Values are expressed as mean $\pm$ standard deviation (SD).

TABLE 3

Standard deviation for subject of age <35 years (n=20) years compared to all subjects (n=32)

SD Age groups	P2 (mm Hg)	P3 (mm Hg)	P4 (mm Hg)	P5 (mm Hg)	P6 (mm Hg)	P7 (mm Hg)	P8 (mm Hg)	PhCI (mm Hg/cm/s)
Dry <35 years	18±27	15±19	9±11	7±4	7±5	8±6	11±6	24±15
All	21±38	20±26	10±12	7±5	6±4	7±6	11±7	30±21
5 mL <35 years	24±23	11±8	12±20	6±3	7±4	9±8	8±6	36±34
All	22±32	14±14	11±16	6±5	8±7	8±7	12±16	30±29
10 mL <35 years	13±12	12±11	13±12	9±8	8±5	6±5	11±5	33±32
All	16±21	18±26	11±10	8±8	10±12	7±6	11±10	34±31

There was no significant difference ($P > .05$) in any of the average standard deviation (SD) values when comparing younger subjects with the larger study cohort. As seen, data from young subjects was not different in magnitude when compared to values across all studied subjects. Values are expressed as mean $\pm$ SD.

TABLE 4

Coefficient of variation for subjects of age<35 years (n=20) compared to all subjects (n=32)

CV	Age groups	P2	P3	P4	P5	P6	P7	P8	PhCI
Dry	<35 years All	0.13±0.17	0.10±0.09	0.08±0.08	0.07±0.05	0.07±0.07	0.08±0.07	0.12±0.10	0.12±0.09
		0.12±0.14	0.12±0.11	0.08±0.07	0.07±0.06	0.06±0.06	0.07±0.06	0.11±0.09	0.11±0.07
5 mL	<35 years All	0.18±0.14	0.10±0.08	0.10±0.12	0.07±0.06	0.06±0.05	0.08±0.08	0.12±0.18	0.17±0.14
		0.14±0.14	0.10±0.09	0.09±0.10	0.09±0.10	0.06±0.05	0.09±0.05	0.11±0.14	0.17±0.12
10 mL	<35 years All	0.13±0.16	0.11±0.11	0.11±0.08	0.08±0.05	0.07±0.05	0.05±0.04	0.15±0.18	0.14±0.09
		0.12±0.13	0.13±0.14	0.09±0.07	0.07±0.05	0.07±0.05	0.05±0.04	0.11±0.15	0.12±0.09

There was no significant difference ($P>0.05$) in any of the average coefficient of variation (CV) values when comparing younger subjects with the larger study cohort. As seen, data from young subjects was not different in magnitude when compared to values across all studied subjects. Values are expressed as mean $\pm$ standard deviation (SD).