**Introduction**

In the greater landscape of medicine, both static and dynamic morphometric variables have been shown to be associated with ‘disease states.’ A prototypical example is Body Mass Index (BMI), which is amenable to intervention and can be conceptualized as dynamic. On the other hand, an individual’s body type (i.e. pear vs apple) is a trait, which is not a readily modifiable ‘static attribute’ associated with cardiovascular disease.

Unlike most other disciplines in medicine, little is known about the “potential” influence of morphometric variables in the development and maintenance of symptoms of urogenital region. This is particularly noteworthy in women where normative data is meager compared to their male counterparts.

The present work is part of a thematically related investigation aimed at characterizing anatomy and neurosensory perception in the female urogenital region; the focus of the present study is on vulvar mucosal distribution.

Surface anatomy of the vulva can best be described in terms of anatomical transition between three different tissues: mucosa (vestibule), non-hairy non-keratinized mucosa (labia minora), and hairy keratinized skin (labia majora) (Fig xx). Because the most common pain complaint in women is that of pain upon contact with the vulvar mucosa (vestibule), we set out to examine the anatomical distribution of the vestibule. This is particularly noteworthy since the etiology of pain of vestibular origin (e.g. vestibulodynia), to date, remains unknown.

Thus, the objective of the present study was to 1) describe vestibular surface measurements using previously described anatomical landmarks (x Citation), 2) investigate potential differences between women with and without vestibular pain, and 3) identify potential clusters of vestibular surface area among women.

**Methods**

**Participants**

Our data was compiled through two institutional review board-approved protocols at the University of North Carolina, Chapel Hill over a seven-year time period (HD ##, PI D. Zolnoun, HD ##, PI D. Zolnoun). Over the course of both studies, 172 women with vestibulodynia and 92 pain-free controls were recruited.

A preliminary eligibility assessment of interested participants was conducted via a telephone interview. A detailed description of the case definition for provoked vestibulodynia and inclusion criteria are described elsewhere (ref). Women who endorsed having unprovoked pain and/or discomfort (such as burning, itching, knifelike, and/or shooting pain) with or without concomitant provoked pain upon contact were excluded from this study. Anatomical measurements were obtained from 172 women with vestibulodynia and 92 pain-free controls were used as a comparison group.

**Examination and Anatomical Measurements**

We measured 8 mucosal sites using the conventional cotton swab with 1 cm markings used in anatomical measurement of pelvic floor prolapse. (xx insert citation- council ). The mucocutanous transition (line delineating the transition between mucosa and non-hairy keratinized epithelium) was marked with a marker prior to measurement (xxx Fig 2 dz made it.) All measurements were rounded up to the nearest 0.5 cm.

The vulvar vestibule is confined entirely within the introitus, and is not entirely a static morphological construct as the introitus has functional sphincter like mechanism. Thus, we conceptualized 6 static and 3 dynamic anatomical sites in this region. The statics sites (described below) were measured first followed by the dynamic sites. We conceptualized the dynamic sites after observing that these measurements changed as a result of the introitus being in a resting state or contracted state. (xxxinsert sophies’ paper xx)

Six out of the 9 anatomical sites (3 on the anterior vestibule and 3 on the posterior vestibule) were determined in reference to the conventional “clock face.” Using an imaginary clock, a total of 12 equidistant sites on the vestibule can be described circumferentially, with positions 12 and 6 corresponding to the anterior and posterior position on the midline (in dorsal lithotomy position), respectively. Thus, sites 10, 12, and 2 are located in the upper vestibule and sites 5, 6 and 7 are located in the lower vestibule (Fig. 1). Furthermore, we used anatomical landmarks in order to standardize the location of these equidistant vestibular sites. Sites 2 and 10 corresponded to the vestibular mucosa measured along an imaginary line through the urethral meatus and parallel to the horizontal plane (x-axis). Sites 2 and 10 were examined in sequence, followed by Site 12, which was located equidistant from the glan clitoris and urtheral meatus measured vertically (y-axis). The posterior sites were selected to be the mirror image of the upper three vestibular sites. Thus, 6 o’clock is the most posterior position on the vestibule and is equidistant from the 5 o’clock position on the right (subject’s left) and the 7 o’clock position on the left (subject’s right). Following the examination of the anterior vestibular sites (2, 10, and 12), the posterior vestibular sites were examined in the following sequence: 5, 7, and 6.

In addition to the above ‘static anatomical landmarks,’ we conceptualized and measured three dynamic measures of the introitus (Fig x): 1) the vertical distance between the 2 o’clock and 5 o’clock positions on the left side (subject’s right), 2) the vertical distance between the 10 o’clock and 7 o’clock positions on the right side (subject’s left), and 3) the “functional” distance in the 6 o’clock position which was the distance from the hymeneal ring to the non-hairy keratinized epithelium (along z-axis). (xxx dz/ need ot make a new photos with all these lines and make reference, fig xxx). All the measurements were taken by the same examiner (DZ).

**Data Analysis**

Statistical analysis was performed using STATA version xx. The two groups (vestibulodynia and pain free control) were compared with respect to each variable of interest. These characteristics include age, race, education, parity, marital status, duration of pain, and xxx. The null hypothesis of no difference between the two groups was tested using Fisher’s exact test for categorical variables and Student t-test for continuous variables. A p-value of <0.05 was deemed significant.

The ratio of the anterior to posterior vestibule was calculated by obtaining the mathematical average of three static vestibular sites on the upper (2, 10, and 12) and lower vestibule (5, 6, and 7), respectively.

In order to identify potential of anatomical measure, a cluster analysis was performed. First, measurements for sites 10 and 2 and then sites 5 and 7 for each participant were averaged to yield an anterior and posterior vestibule. Next, a K-means clustering algorithm (with Euclidean distance measure) was used to group participants into vestibular types based on these measurements. The number of clusters, k, was set to equal 3 based on clinical observation. Other specifications of k were also tested, but the 3-cluster model fit the data most effectively.

**Results**

Our cohort primarily consisted of educated, white women in their thirties.

With the notable exceptions of marital status and race, the subgroups of women with and without vestibulodynia were similar (Table 1).

The anterior vestibular surface (defined as the mathematical average of measures obtained at sites 2, 10, and 12) was larger than the posterior vestibular surface (defined as the mathematical average of measures obtained at sites 5, 6, and 7) in all the women regardless of their case status. (xxx I like ending)

The ratio measure of the anterior to the posterior vestibule, however, showed three morphometric patterns (discussed in a later section). This ratio measure, however, was not different between the two groups of women with or without vestibulodynia.

We similarly did not observe any differences in 6 static measures of the vestibule (Table 1). Unlike static measures, however, we did find significant differences between the two groups in dynamic measures of the vestibule, especially at the 6 o’clock position. Specifically, women with vestibulodynia had a shorter functional length at the 6 o’clock position of the introitus (1.91 cm +SE?? vs 2.21 cm +SE?, p = 0.023) compared to the pain free comparison group (Table 2). The remaining two sites (along the y-axis) were less robust and did not quite reach statistical significance (p= 0.086) in two-tailed tests, but were significant in the expected direction in one-tailed tests (p= .043), with women in the vestibulodynia group having shorter distances (see discussion).

We identified three distinct classes of the vestibule using cluster analysis, hereon called Class I, II, III. Class I consisted of women with the largest difference between the anterior and posterior vestibule as demonstrated by the largest ratio. In contrast, women in class III represented those with the smallest difference between the anterior and posterior vestibule. The most common anatomical variation (representing nearly 70% of our population) consisted of women with anatomical measures intermediate between the two groups (class II). Figures 3 -5 represents prototypical examples of women falling into each of the three groups.

**Discussion**

In this initial study we described feasibility of measuring vestibular surface with deference to static and dynamic elements of the anatomy. In addition, we confirmed the findings of other investigators with respect to morphometric changes that may occur in the context of disease state, namely vestibulodynia (x sophies paper). Lastly, we identified three distinct clusters of vestibular surface area with respect to relative dominance of the posterior vs. anterior vestibule.

Because one investigator primarily performed all the measurements we have limited data on reliability and reproducibility. Furthermore, we used a relatively “crude” cotton swab to measure a small surface area, leading to a limitation in precision. All measurements were rounded up to the nearest 0.5 cm; measurements below 0.5 cm were reported as 0.5 cm. This is particularly relevant in measuring sites in the posterior vestibule (sites 5, 6, and 7) where values below 0.5 cm were frequently encountered. We also used ‘calculated’ landmarks for measuring the posterior vestibular sites in that these three sites were mirror images of their respective counterparts on the anterior vestibule. Despite our attempt at a standardized approach to measurement, measuring the posterior vestibule posed additional challenges in that the muco-cutanous transition (line delineating the vulvar mucosa from the keratinized non-hairy skin) in some women was not readily visible. This was particularly challenging following vaginal birth, though the distribution of vaginal birth did not differ among subgroups. It is unlikely that the above-mentioned methodological and measurement variability significantly altered our findings specifically with respect to the 6 static measures of the vestibule. In contrast to static measures, however, dynamic measures of the vestibule were affected by systemic distortion due to our measurement methodology discussed below. Lastly, our findings may not be generalized to non-Caucasian women; our cohort was primarily Caucasian (80%).

Introital contraction of various severities is a sine qua non finding upon examination of women with vestibulodynia. Conventionally thought to be secondary in nature and in response to inflammation of the overlying mucosa and guarding, there is little disagreement about the contribution of muscle contraction (voluntary or otherwise) in perpetuating the cycle of pain and disability. Because we used a wooden cotton swab we were unable to consistently position the swab in an anatomically congruent manner because of this contraction. However, our methodology and findings are consistent with findings of other investigators with respect to perineal contraction. Consistent with this clinical observation, using a one sided t-test illustrated a more robust association. Informed by our findings, we are presently collecting data using a more nuanced bed side assessment methodology (xHD – current grant number) in order to confirm and expand upon this work.

The lack of association between static measures of the vestibule and a vestibulodynia case status was not surprising. While various degrees of pain and inflammation are reported by these patients, a clinical mucositis with erosive changes and associated denudation in muco-cutanous transition (as commonly seen in inflammatory conditions such a lichen planus) is uncommon.

In addition to the static and dynamic measures of the vestibule, we identified three distinct classes of the vestibule among women. While the distribution of the vestibule between the anterior and posterior region did not differ among the cases and the controls, these patterns may have unknown clinical implications with regards to patients’ chief complaints and presenting symptoms.

Because these anatomical measurements were conducted in the course of a larger study aimed at identifying neurosensory precept, we were able to make several clinical observations, which will be explored in greater detail in future manuscripts. For example, women with larger ratios (relatively greater surface area in the anterior component) tended to report a higher degree of co-morbid urinary symptoms (urgency and dysuria) among women with vestibulodynia. We speculate that this may, in part, reflect a referral pattern from the underlying muscle. Alternatively, if the underlying mechanism of pain was in fact mucosal in origin and given the close proximity of the urinary meatus (and shared embryologic origin) it will be reasonable to expect “urinary-related symptoms” which may be exacerbated in the context of daily toileting and grooming. Regardless, this raises interesting questions with respect to the underlying mechanism and contribution of anatomical factors in the presenting symptoms in a number of elusive urogenital pain disorders – with high comorbidity- such as vestibulodynia and interstitial cystitis in women.

We believe that our methodology and conceptualization of anatomy can provide additional data points in research and clinical care of women with urogenital symptoms. Armed with normative data of the vestibule’s surface area one can potentially put in context a patient’s symptomatology. Furthermore, this conceptualization allows for objective assessment of therapeutic interventions such as physical therapy by means of objectively tracking therapeutic response using a bed side assessment methodology and noting changes in dynamic measures (among other variables) of the vestibule. Alternatively, static measures of the vestibule and its respective changes with intervention can serve as a simple bed tool to capture treatment responses. Discussion of the sensory precept of the vestibule using this methodology will be explored in future manuscripts.

In the era of individualized medicine we are in dire need of foundation level knowledge in order to understand the interplay between various domains that may be operative in the genesis of pain and reported symptoms in the urogenital region such a morphology. In this manuscript we discussed our finings with respect to some anatomical factors that may affect disease manifestation and symptoms reported while providing normative data on the vestibular surface area. This work needs to be replicated and expanded upon by other colleagues if we hope to be able to shift the state of science and clinical care of these patients from that of ‘regional’ to one of mechanism-based conceptualization.