Vaginal wall changes in muscles and connective tissues after vaginal birth

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Abstract: Introduction: The vagina is central to pelvic organ support such that a normally supported vagina will resist downward descent of the uterus, urethra, bladder, and rectum. If the structural and mechanical integrity of the vagina and/or the structures that support it are compromised, pelvic organ prolapse may occur. Maternal birth injury subsequent to vaginal delivery is considered the greatest risk factor for the development of prolapse. **Objective**: to determine the effect of stretching and vaginal distention involved in vaginal delivery on the structure of muscles and connective tissue of the vaginal wall. **Study Design**: pilot retrospective case-control study. **Aim of the work**: reduce the risk of birth injuries and preserve the vaginal tissues from being redundant and unhealthy. **place and duration:** Department of Obstetrics & Gynacology, faculty of medicine-Azhar University from march to july2012 . **Patient & Method:** 40 women aged 20 to 35 years were enrolled in this study subdivided into two groups ,study&control each consists of 20 women. The study group includes women who gave birth through NVD, and control group includes women who gave birth through C.S. All women were subjected to full history taking with special regards to (age, parity and mood of delivery) and full clinical examination (general, abdominal and local examination). A full-thickness vaginal specimen was obtained from all women and all specimens were fixed in formalin (10%) for 24h. then Cross sections of the formalin-fixed, paraffin-embedded tissues will be obtained for histopathology study using heamatoxylin and eosin. Special cytochemistry Trichrome staining will be used for evaluation of fibrocollagenous stroma and vaginal wall smooth muscle changes. **Results:** . Redundant vaginal wall was noticed in 8 out of 20 NVD cases (40.0 %). vaginal redundancy was noticed more frequently with increased parity. We found that vaginal redundancy was 40% in women with vaginal delivery as compared to 0.0% in women with cesarean section (C.S) (p = 0.002). All women with redundant vagina have altered vaginal tissue histomorphology in the form of: Minimal total vaginal collagen, loose fibrocollagenous stroma, severely affected muscle integrity (muscle bundles are smaller, splitted, fragmented and disorganized compared with control group). **Conclusion**: Vaginal parity was associated with alternation of the vaginal tissue histomorphology that affect the vaginal condition to be redundant.

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Keyword : histopathology of vaginal wall after vaginal birth, pelvic organ prolapse

1. Introduction

Women have been expected to pay a lifelong price for a baby's birth. Pelvic floor dysfunction, in the form of a type of hernia termed pelvic organ prolapse (including cystocele, rectocele, and/or uterine prolapse) and urinary and fecal incontinence are considered inevitable sequelae for some women who experience injuries during birth (***James and John, 2009*).** Pelvic organ prolapse is one of these sequelae which is a common problem for women with significant psychological, social, and financial implications. Whereas many factors appear to incite or promote the progression of prolapse, the two major risk factors for pelvic organ prolapse are history of vaginal delivery and aging ***(Linceet al., 2012)***. Although cesarean delivery does not completely protect the pelvic floor from the adverse effects of pregnancy, it is universally agreed that vaginal delivery has a deleterious impact on the development of pelvic organ prolapse in women, and this impact may not be of clinical significance until decades after delivery. ***(Schwertner-Tiepelmann et al.,2012)***

Vaginal delivery is also the single most important risk factor for developing stress urinary incontinence, and the need for treatment increases inexorably with advancing age (***James and John, 2009*).** The mechanisms by which childbirth leads to failure of pelvic organ support, however, are not well understood (***Rahnet al., 2008***).

Female reproductive organs are rich in elastic fibers that turn over slowly in most adult tissues but undergo massive remodeling in the reproductive organs through pregnancy and birth as during pregnancy and parturition, tissues in the reproductive tract undergo profound changes that include breakdown and resynthesize of elastic fibers. An inability to rebuild the elastic fiber network might be anticipated to produce some structural and functional deficits in these tissues (***Xiaoqing et al., 2006***). And it seems logical that the trauma of physical stretching

and vaginal distention involved in vaginal delivery may play a role in turnover of connective tissue components of the pelvic floor (***Rahn et al., 2008***).

Aim of The Work

The objective of this study is to determine the effect of stretching and vaginal distention involved in vaginal delivery on the muscles and connective tissue of the vaginal wall.

2.Patients & Methods

Patients: This is a pilot retrospective case-control study that was conducted on 40 women aged 20 to 35 years. They were randomly selected from women undergoing surgery or labor in the department of Obstetrics and Gynecology of Al Zahraa university hospital.

The sample was divided into two groups as follow:

-The study group consists of 20 women who had delivered vaginally.

-The control group consists of 20 women who had delivered by cesarean section (C.S)

Inclusion criteria:

-Women who had delivered vaginally for the study group and women who had delivered by C.S for the control group.

-Women aged 20 to 35 years to exclude the effect of age on the tissues that will be used in the study.

Exclusion criteria:

-Women aged more than 35 years.

-Women with already present any connective tissue disorder.

Methods: All women involved in this study (study and control groups) were subjected to the following:

-Full history including (age , parity , mode of delivery)

-Full general examination to exclude any associated connective tissue disorder.

-Local examination to assess any associated pelvic floor dysfunction.

-Pelvic Organ Prolapse Quantification (POP-Q) examination, and vaginal wall with stage I or more was considered redundant.

-A written consent was taken from them after informing them about the procedure and importance of the study.

-Taking full-thickness vaginal specimen which will be fixed in formalin (10%) for 24hrs. Cross sections of the formalin fixed, paraffin-embedded tissues will be obtained for histopathology study using heamatoxylin and eosin (H&E). Special cytochemistry Masson's Trichrome (MT) staining will be used for evaluation of total collagen, fibrocollagenousstroma and vaginal wall smooth muscle changes.

Semi-quantitative method was designed to assess pathologic changes on Trichrome stained sections as follow:

Connective tissue :

-Abundant compact collagenous stroma: Score 3

-Moderate content: Score 2

-Loose pale stroma: Score 1

Smooth muscle component and integrity:

-Bulky compact muscle layer (intact): Score 3

-Disintegrated muscle bundles (moderately affected): Score 2 (Where the muscle fiber were separated by intervening collagenous stroma)

-Atrophic and / or lost muscle (severely affected): Score 1

Pelvic Organ Prolapse Quantification (POP-Q)

•This system contains a series of site-specific-measurement of woman's pelvic organ support.

•Prolapse in each segment is measured relative to the hymen, which is a fixed anatomic landmark.

•Six points are located with references to the plane of the hymen (zero reference point), measurements above (cranial to) the hymen are presented as negative numbers and measurements below (caudal to) the hymen are positive. These 6 points are as follow:

Aa: The position of the distal anterior vaginal wall, 3 cm proximal to the external urethral meatus.

Ba: The most distal position of the remaining anterior vaginal wall above point Aa.

C: The most distal edge of the cervix or vaginal cuff (if cervix is Absent).

D: The position of the posterior fornix (omitted if cervix is absent).

Ap: The position of the posterior vaginal wall, 3 cm proximal to the hymen.

Bp: The most distal position of the posterior vaginal wall above point Ap.

Staging system of pelvic organ support:

0: No prolapse: All points are 3 cm above the hymen

I: The most distal portion of the prolapse is >1 cm above the distal level of the hymen.

II: The most distal portion of the prolapse protrudes to or beyond 1 cm above the hymen but not more than 1 cm below the plane of the hymen.

III: The most distal portion of the prolapse > 1 cm below the Plane of the hymen, but protrudes no further than 2 cm less than the total vaginal length in centimeters.

IV: Complete evertion of the total length of the lower genital tract.

Statistical methods:

Microsoft® Excel® (version 2007) and SPSS® for Windows® version 15.0 used for data presentation and statistical analysis. Outcomes of both groups will be compared using t-test (for quantitative parametric measures), Mann-Whitney's U-test (for quantitative

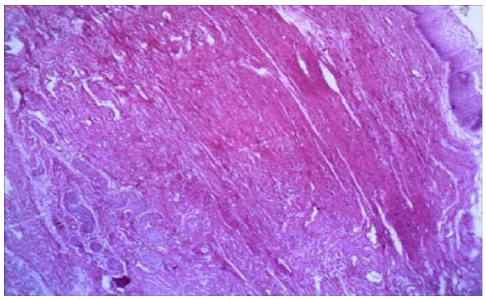
non-parametric measures) and chi-squared and Fischer's Exact tests (for categorical measures). Pearson's correlation coefficient will be used to estimate association between metric variables. Significance level will be set at 0.05.

3. Results

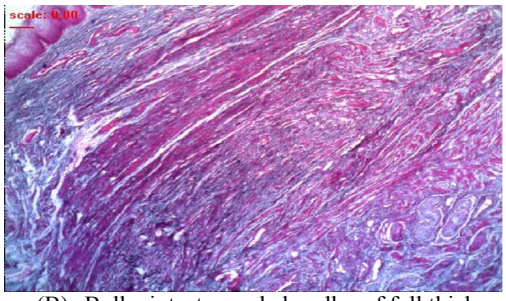
The study included 40 patients. Their ages were ranged from 20 to 35 years with mean 29.5 ± 2.39 Normal vaginal delivery (NVD) was recorded in 20 patients (50% of all cases) and cesarean section (C.S) in the other20 cases. Redundant vaginal wall was noticed in 8 out of 20 NVD cases (40.0 %). **Table 1** As regarding parity vaginal redundancy was noticed more frequently with increased parity. **Table 2**

Histopathology:

Normal vaginal canal was lined by an average layer of non keratinized stratified squamous epithelium with average glycogen content of the superficial layers. Lamina propria showed variable thin wall vessels and loose connective tissue. Compact smooth muscle bundles with circular arrangement was noticed. It is surrounded by nerve plexus and many nerve bundles were seen between the bundles**.** Thick vessels were immersed in an average collagenous connective tissue **(Fig. 1)**. This picture was noticed mainly in women with C.S (control group).



(A) Bulky intact muscle bundles (M) of full thickness surrounded by nerve bundles (control) H&E X 40.



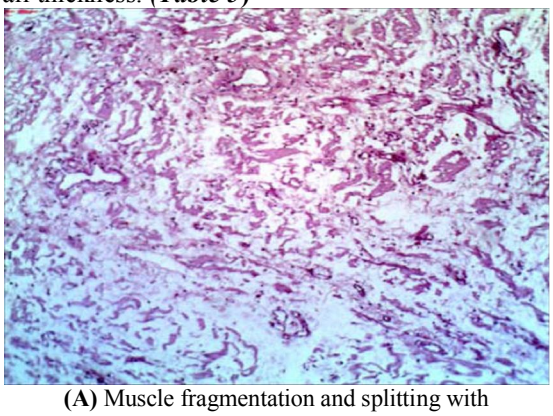
(B) Bulky intact muscle bundles of full thickness (control) MT X 40

Fig. 1: Normal vaginal wall histomorphology.

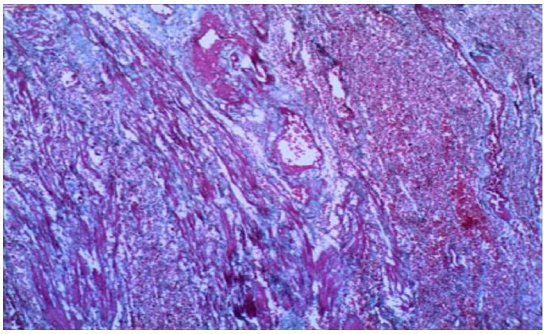
Different pattern of vaginal wall histomorphology changes were noticed with redundant vagina that was noticed only in women with vaginal delivery **(Fig. 2)** in the form of:

- Minimal total vaginal collagen. ***(Table 3)***
- Loose fibrocollagenous stroma involving all vaginal wall thickness. ***(Table 5)***
- Severely affected muscle integrity (muscle bundles are smaller, splitted, fragmented and disorganized compared with control group.) ***(Table 8)***
- Few nerve bundles.

A B



(A) Muscle fragmentation and splitting with intramuscularhemorrhage (H & E X100)



(B) Muscle fragmentation and splitting with intramuscular hemorrhage. Fibrocollagenousstroma appeared blue (arrow) MTX100

Fig. 2: Histomorphology of redundant vaginal wall.

The damaging effect resulting from the stretching of vaginal tissue due to passage of fetal head was noticed to be increased with increasing the number of vaginal births **(Figs 3 , 4) (Tables 7, 10).**

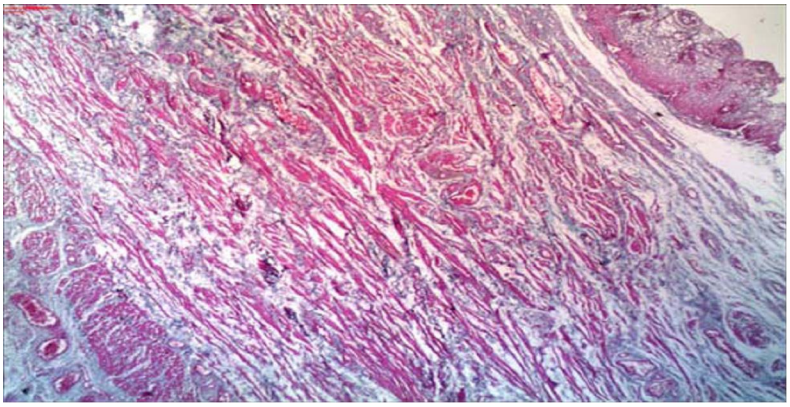


Fig. 3 : Section in vaginal wall with NVD , p2: Average smooth muscle bundles with moderate intervening loose fibrocollagenousstroma. Some bundles were over stretched and others were fragmented. Deep muscle bundles were intact. Overlying vaginal mucosa was noticed (arrow) (MT X 40).

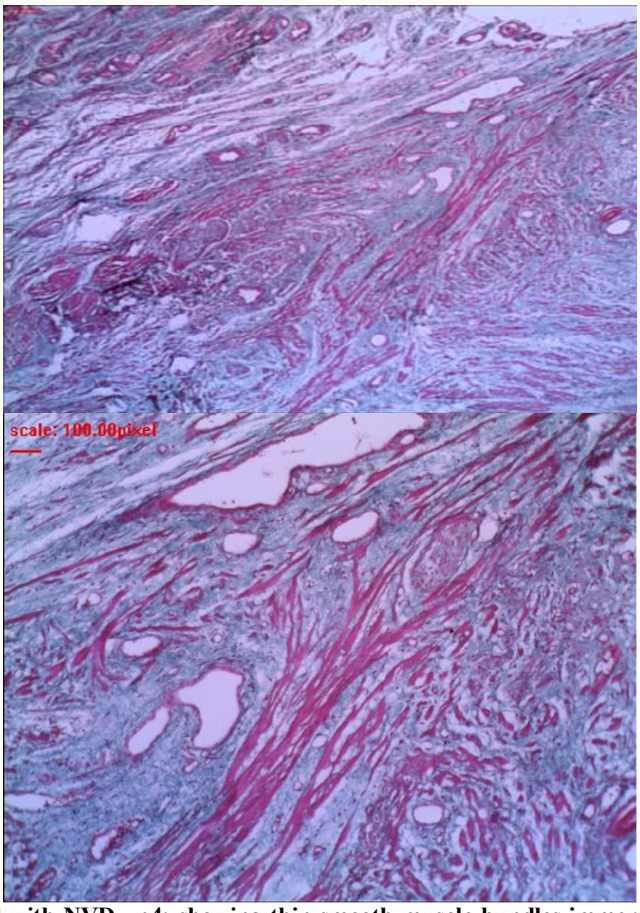


Fig 4: Section in vaginal wall with NVD , p4: showing thin smooth muscle bundles immersed in loose connective tissue. Some bundles were over stretched and others were fragmented. Some vascular spaces were thrombosed (A: MT X 40 and B: MT X100).

Table (1): Relation between mode of delivery and vaginal condition.

		Vaginal condition				Total	Fisher Exact test	P- value
		Normal		Redundant				
		No	%	No	%			
Number of births	1	5	100.0%	0	0.0%	5	20.503	<0.001
	2	4	100.0%	0	0.0%	4		
	3	2	50.0%	2	50.0%	4		
	4	1	25.0%	3	75.0%	4		
	5	0	0.0%	3	100.0%	3		

This table shows that the mode of delivery has significant effect (*P*-value = 0.002) on the vaginal condition.

Table (2): Relation between number of vaginal births and vaginal condition.

		Vaginal condition				Total	Fisher Exact test	P- value
		Normal		Redundant				
		No	%	No	%			
Number of births	1	5	100.0%	0	0.0%	5	20.503	<0.001
	2	4	100.0%	0	0.0%	4		
	3	2	50.0%	2	50.0%	4		
	4	1	25.0%	3	75.0%	4		
	5	0	0.0%	3	100.0%	3		

This table shows that number of vaginal births has significant effect (*P*-value <0.001) on the vaginal condition.

Table (3): Relation between the amount of total vaginal collagen and vaginal condition.

		Vaginal condition				Total	Fisher Exact test	P- value
		Normal		Redundant				
		No	%	No	%			
Total collagen	Normal	11	34.4%	0	0.0%	12	17.939	<0.001
	Moderate	16	50.0%	0	0.0%	16		
	Minimal	5	15.6%	8	100.0%	12		
Total		32	100.00%	8	100.00%	40		

This table shows that the amount of total vaginal collagen has significant effect (*P*-value <0.001) on the vaginal condition.

Table (4): Relation between mode of delivery and the amount of total vaginal collagen.

		Mode of delivery				Total	P- value
		NVD		C.S			
		No	%	No	%		
Total collagen	Normal	0	0.00%	11	100.00%	11	<0.005
	Moderate	7	43.80%	9	56.20%	16	
	Minimal	13	100.00%	0	0.00%	13	

This table shows that the mode of delivery has significant effect (P-value <0.005) on the amount of total vaginal collagen.

Table (5): Relation between the state of vaginal Fibrocollagenous stroma and vaginal condition.

		Vaginal condition				Total	Fisher Exact test	P- value
		Normal		Redundant				
		No	%	No	%			
Fibrocollagenous stroma	Abundant	10	31.3%	0	0.0%	10	19.907	<0.001
	Moderate	18	56.3%	0	0.0%	18		
	Loose	4	12.5%	8	100.0%	12		
Total		32	100.00%	0	100.00%	40		

This table shows that the state of vaginal Fibrocollagenous stroma has significant effect (P-value <0.001) on the vaginal condition.

Table (6): Relation between mode of delivery and the state of vaginal fibrocollagenous stroma.

		Mode of delivery				Total	P-value
		NVD		C.S			
		No	%	No	%		
Fibrocollagenous stroma	Abundant	0	0.00%	10	100.00%	10	<0.005
	Moderate	8	44.40%	10	55.60%	18	
	Loose	12	100.00%	0	0.00%	12	

This table shows that the mode of delivery has significant effect (P-value <0.005) on the state of vaginal Fibrocollagenous stroma.

Table (7): Relation between number of vaginal births and the state of vaginal fibrocollagenous stroma.

		Fibrocollagenous stroma						Total	P-value
		Abundant		Moderate		Loose			
		No	%	No	%	No	%		
Number of births	1	5	100.0%	0	0.0%	0	0.0%	5	<0.005
	2	0	0.0%	4	100.0%	0	0.0%	4	
	3	0	0.0%	2	50.0%	2	50.0%	4	
	4	0	0.0%	1	25.0%	3	75.0%	4	
	5	0	0.0%	0	0.0%	3	100.0%	3	

This table shows that number of vaginal births has significant effect (P-value <0.005) on the state of vaginal Fibrocollagenous stroma.

Table (8): Relation between the state of vaginal muscles and vaginal condition.

		Vaginal condition				Total	Fisher Exact test	P-value
		Normal		Redundant				
		No	%	No	%			
Muscle state	Intact	18	56.3%	0	0.0%	18	16.371	<0.001
	moderately affected	7	21.9%	0	0.0%	7		
	severely affected	7	21.9%	8	100.0%	15		
Total		32	100.00%	8	100.00%	40		

This table shows that the state vaginal muscles has significant effect (P-value <0.001) on the vaginal condition.

Table (9): Relation between mode of delivery and the state of vaginal muscles.

		Mode of delivery				Total	P-value
		NVD		C.S			
		No	%	No	%		
Muscle state	Intact	0	0.00%	18	100.00%	18	<0.005
	moderately affected	5	71.40%	2	28.60%	7	
	severely affected	15	100.00%	0	0.00%	15	

This table shows that the mode of delivery has significant effect (P-value <0.005) on the state of vaginal muscles.

Table (10): Relation between number of vaginal births and the state of vaginal muscles.

		State of vaginal wall muscles						Total	P-value
		Intact		Moderately affected		Severely affected			
		No	%	No	%	No	%		
Number of births	1	0	0.0%	4	80.0%	1	20.0%	5	<0.005
	2	0	0.0%	2	50.0%	2	50.0%	4	
	3	0	0.0%	1	25.0%	3	75.0%	4	
	4	0	0.0%	0	0.0%	4	100.0%	4	
	5	0	0.0%	0	0.0%	3	100.0%	3	

This table shows that number of vaginal births has significant effect (P-value <0.005) on the state of vaginal muscles.

4.Discussion

Maternal birth injury subsequent to vaginal delivery is considered the greatest risk factor for the development of prolapse. ***(Feola et al., 2010)***

However, no previous studies performed a comprehensiveevaluation of the effect of birth injury on the human vaginal tissue by correlating the gross anatomical findings and alterations in tissues histomorphology.

In this study, we examined the impact of vaginal delivery on the human vagina usinghistomorphologic outcomes. The most important findings of the study were that vaginal parity was associated with alternation of the vaginal tissue histomorphology that affect the vaginal condition to be redundant.

In this study we assumed that the vaginal delivery has a great effect on the composition and structural organization ofthe dense connective tissue and muscle layers of the vagina which are the major determinants of the vaginal condition.

We found that vaginal redundancy was 40% in women with vaginal delivery as compared to 0.0% in women with cesarean section (C.S) ($p = 0.002$). ***(Table 1)***

It is obvious that vaginal dilatation produces more of vaginal wall redundancy. Were there is redundancy the degree of damage ***(Fig.1)*** of the vaginal wall structures is: Minimal total vaginal collagen ($P < 0.001$). ***(Table 3);*** Loose fibrocollagenousstroma ($P < 0.001$). ***(Table 5);*** Severely affected muscle integrity (muscle bundles are smaller, splitted, fragmented and disorganized compared with control group.) ($P<0.001$). ***(Table 8)*** Minimal nerve bundles.

As we have seen in ***(Tables 4,6,9)*** all alternations of the vaginal tissue histomorphology that associated with redundant vagina are present only in women that gave birth through NVD and not in women delivered through cesarean section (C.S), ($P < 0.005$). We also found that increased vaginal parity has a significant effect on the vaginal condition ($P < 0.001$). ***(Table2)*** and on vaginal tissue histomorphology ($P < 0.005$). ***(Tables 7, 10).***

Our study has some limitations. First, we used human tissues that are associated with some problems related to obtaining tissue biopsy samples in humans that include inconsistent tissue quality from manipulation during procurement, and variable exposures such as hormone use and physical activity.

Second, pregnancy itself without vaginal dilatation can produce damage. Finally we can't provide a definitive relationship between the changes in the vaginal tissue histomorphology that are observed with vaginal parity and those changes that are seen in patients with POP. Therefore we can speculate that women with redundant vagina would be susceptible to the progression and development of prolapse overtime by comparing the finding of our study as regard to the changes in vaginal wall histomorphology in women with redundant vagina with that of the studies which have demonstrated the alternation of the vaginal tissue histomorphology in patients with POP as ***(Word et al., 2009)***.

It is obvious that the vagina undergoes a process of effacement and dilatation as the cervix to accommodate delivery of fetal head.

It is safe to speculate that passage of the fetal head affects the vaginal wall structure by overstretching of vaginal wallleading to splitting, fragmentation and disorganization of muscle bundles, injury of nerve bundles and disruption of supporting connective.

Conclusion

Vaginal parity was associated with alternation of the vaginal tissue histomorphology that affect the vaginal condition to be redundant.

Vagina undergoes a process of effacement and dilatation as the cervix to accommodate delivery of fetal head.

Recommendations

We recommend gradual vaginal distention , avoiding the instrumental delivery and performing pelvic floor muscle exercise during pregnancy and after labor to reduce the risk of birth injuries and preserve the vaginal tissues from being redundant and unhealthy.

However, more studies will be required in order to confirm the findings of our study.

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