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EVALUATION OF METHYLATION STATUS OF ESTROGEN RECEPTORS IN CASES WITH ENDOMETRIOSIS

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Abstract

Background:*Endometriosis is characterizedby the existence and growing of functional endometrial tissues, out of the uterine cavity, mainly in the ovaries, pelvic peritoneum and rectovaginal septum. While it is a benign disease, it appearswith the characteristics of malignant, like invasions to adjacent tissue, metastasis to distant positions and recurrence following treatments,****Aim and objectives:*** *The current work aimed to evaluate the methylation condition of estrogen receptor gene in endometriosis. The ultimate objective is to determine any potential role for such receptors as a diagnostic marker for the disease process.****Subjects and methods****: The current work was demonstrated on fifty cases recruited from gynecology clinics of Shatby Maternity Hospital Alexandria University complaining of pain or infertility. On ultrasound examination using 2D ultrasound (mindray) to diagnose endometrioma or pelvic endometriotic nodules. Laparoscopy was done for (30) cases and a biopsy were taken from suspicious endometriotic lesion.* ***Results****: A significant changewas found between cases and controls in terms of methylation index of estrogen receptors where cases values being 0.02-132.9 with mean value12.99 and p value <0.05 and control group being0.14-164.4 with a mean 66.04 and p value>0.05,****Conclusion****: The finding suggests that, Estrogen receptor gene hypomethylation in endometriotic tissue. Progesterone (P4) oppositionis found in endometriosis in general with down regulation of PR-B, as a result of promoter hypermethylation of PR-B.*

Keywords: *DNA Methylation, Endometriosis, Pain, Endometriotic Nodules.*

I. INTRODUCTION

In the earlier20 years, epigenetic mechanisms weredefined as significant factors in the complex human disorders' developments, involvingtumor, and neuro-degenerative, neurologic and auto-immune since they couldmodify gene expressionsindependent on DNA sequence diseases. Fluctuations in chromatin configurationestablish the base of epigenetic influences changes. **(Ballestar et al., 2011)**

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Newindicationshows that endometriosis, enigmatical diseases in which endometrial-similar tissuesarefound out of the uterus, is as well an epigeneticdisease. This suggestion was founded on the anomalous DNA methylation pattern detected inthe promoter areas of definite genes and the advancedexpressing levels of DNMTs in endometriotic lesions relative toordinary endometrium.**(Wang et al., 2012)**

This epigenetic alteration is catalyzed by DNA methyltransferases (DNMTs), which adjoin a methyl-group to the carbon-5 of cytosines that are trailed by a guanine. Consequently, DNA methylationshappensnearcompletely in the background of a 5'-CpG-3' dinucleotide, principally in condensedCpGareasnamed asCpG-island. Promoter-definiteCpG island hypomethylationis recognized to be accompanying with gene silencing because of their transcriptsuppression effects.**(Illingworth et al., 2009)**

Additionally, hypomethylationof the PGR genes, principally at promoter B, was detected as well in the epithelial components of endometriotic lesions, with resultingreduction in the expressions of its transcripts.

Total, steroid hormone receptor genes exhibitcomplexed patterns of regulationsincluding multi-promoters and substitute mRNA isoform. The ESR1 geneshave two proximal promoters (transcripts A and B) placed within ~2 kb of the translations startinglocation and an up-stream promoter C.**(Grandien et al., 1997)**

Correspondingly, the PGR geneshave two alternate transcripts structured by 2 promoter definitelocations for the PGRA and PGRB isoforms, which are accompanying with well-definedCpG-island.

The ESR2 gene as wellexists alternatively linked transcript variant and separate promoters structured by DNA methylations, though not accompanying with the existence of classic CpG-island. Possibly, abnormal DNA methylationscoulddisturb the influences of hormone steroids because ofvariations in the expressing level of its receptors and mightaffect the origins and progressions of endometriosis.**(Hirata et al., 2001)**

It wasestablished that DNA methylationscould interfered with protein-DNA interactions, employment of histone deacetylases, and the inductions of chromatin condensationsrequired for genesdeactivation. **(Hirata et al., 2001)**

Methylation couldoverlap straightly with the DNA linkingtodefinite transcription factors. As well, a number of methyl-CpGlinking proteins are revealed to bonded to methylated DNA and modify its DNA conformations, thus influencing the bond of several transcription regulators. These molecular changesaccompanying with the methylations of the ESR2 promoter might be reliable for its repression in endometrial stromal cells.**(Vincent et al., 2010)**

Correspondingly, the ESR2 expressionsin the stromal cells of endometriosis might be structured by factors otherwise methylations. For instance, sequence analyzing of the 50-flanking area of the ESR2 promoter 0N hadrevealed the existenceof someconsent transcription factor linkinglocations and cis-regulatory element.**(Vincent et al., 2010)**

This was the primaryexpression of methylation-related mechanismsaccountable for extraordinarilyraised ESR2- levels in endometriosis. This resultmighthavemany clinical functions. For the reason that the methylation of a definite gene could be discovered in DNA from the diagnosing biopsies, ESR2 methylation conditioncan be a possiblyuseful assistant to morphologic principles for the diagnostics of

endometriosis. Additionally, studying ESR2methylations in endometriotic lesions mightrecognizecases who are applicants for treatments with ESR2-choosy composites.**(Hummelshoj et al., 2006)**

II. PATIENTS

The current work was demonstrated on fifty cases recruited from gynecology clinics of Shatby Maternity Hospital Alexandria University complaining of pain or infertility. On ultrasound examination using 2D ultrasound (mindray) to diagnose endometrioma or pelvic endometriotic nodules. Laparoscopy was done for (30) cases and a biopsy were taken from suspicious endometriotic lesion. Endometrial biopsy taken from (20) control cases by endometrial sampling.

The cases were subdivided into 2 groups: Group (a): study group (n=30) diagnosed with any stage of endometriosis. **Group (b)**: control group (n=20) control group: women with no endometriosis.

Exclusion criteria: Chronic medical conditions; e.g hypertension, diabetes, Postpartum (up to 6 weeks), Contraindication or no wish for surgery, History of pelvic inflammatory disease and Patients on hormonal therapy, antiprolactin drugs and thyroid drug.

III. METHODS

This work was accepted by ethical committee of the faculty of medicine, Alexandria University.

All cases signed a well-informed written agreement to declare their agreement to be comprised in this work.

The two groups were subjected to:

- **History:** Personal, medical, surgical, family and detailed present history Including: Age, date of marriage, gravidity, parity, last menstrual period, date of menarche, regularity of the cycle, complaints; dysmenorrhea, dyspareunia and infertility.
- **Complete general and local physical examination.**
- **Investigation**:Routine laboratory investigations. Complete blood picture, fasting blood sugar and partial thromboplastin time.2D transvaginal ultrasound using to diagnose endometriotic cyst (with low-level innerechoes, and occasionally thick septations, thickened wall, and echo-genic wall foci.) or pelvic nodule.MRI pelvis and abdomen with or without contrast for confirming the diagnosis of the pelvic nodule.

Laparoscoy was done for study group and multiple biopsies were taken from suspicious peritoneal, ovarian or uterine lesions. Endometrial biopsy taken from (20) control cases by outpatient endometrial biopsy pipelle.

Sampling

DNA extraction: Genomic DNA was obtainedfrom tissue specimensvia the commercially DNA extracting kitsQIAamp DNA; (Qiagen, Hilden, Germany).

DNA bisulphite conversion: The DNA obtained from tissue specimenswere bisulphite converted for further methylation analyzing using EpiTect Fast DNA Bisulfite Kits (Qiagen, Hilden, Germany).

Carrier RNA: Carrier RNA increasesbonding of small amounts of DNA to the spin column membranes. 310 µlRNase-free water were supplemented to the lyophilized carrier RNA (310 µg) to attain 1 µg/µlsolution. The carrier RNA was dissolved carefully by vortexing. The dissolved carrier RNA was split into 50 µl aliquots and kept at –20°C.

- **Methylation analyzing by SYBR green-based quantitative methylation specific PCR (MSP)**

Principle

Quantitative methylation specific polymerase chain reaction (qMSP) SYBR Green-based quantitative methylation polymerase chain reactionswereaccomplishedafterward the bisulfite treatments on the denaturizedgenomic DNA.

SYBR-Green is definite for dual-stranded DNA and fluoresces when bound to the amplified dual-stranded PCR products, soallowingstraight quantifying of amplified DNA with nomarked probes.

β-actin (the reference gene, internal control, normalizer)

β-actinserved as a reference controls to normalize the quantity of methylated targets alleles identified(threshold cycle (C_t) values).[93] A primer pair equivalent to a definite β-actin sequence was selected. Within this β-actin sequence no CpGlocations are existing. Therefore, the cytosines are continuously un-methylated and consequently will continuously be transformed to uracil afterward bisulfite treatments.

Controls

Control reactions were performed to confirm that the PCR primers are exact for detectingthe methylated bisulfite transformed DNA. Entirely methylated DNA (methylated human control DNA, bisulphite converted); (EpiTect®PCR Control DNA set, Qiagen, Germany) was involved as a positive control in every set of reactions besides a negative control sample with no DNA.

Protocol of amplification of ESR1Agene

The specimens were utilized with a 12-min, 95˚C, activating step, 40 cycles of a 1-minute, 94˚C denaturizing step, a 1-min, 64˚C annealing step, and a 1-min, 72˚C extending step, tailed by a melting-curve step.

Protocol of amplification of -actin gene

The samples were run with 3-minutes, 95˚C, activation step, 40 cycles of 5-seconds, 95˚C denaturizing step, a 10-seconds, 65˚C annealing step, and a 20-seconds, 72˚C extending step, tailed by a melting-curve step.

Calculation of ESR1A gene methylation level (relative comparative Ct method)

The basisof SYBR green-builtquantitation was founded on the concept that the luminous signalsproducedby the intercalation of SYBR Green color into enhanced DNA is straightlyproportionate to the quantity of enhanced DNA. The recorded fluorescent signalswereconverted into the Ctvalue, which a representative of the quantity of PCR-amplified DNA product.

Statistical analysis of the data

Data was analyzed via the windows-based IBM-SPSS programV-20.0. (Armonk, NY: IBM Corp) Qualitative data wasintroduced in the form of numbers and percents. The Kolmogorov-Smirnov testing was employed to find the normality of distributing. Quantitative data wasintroduced in the form ofrange (min and max), mean, standard deviation (SD), median and interquartile ranging (IQR). Significancy of the results was considered at thelevel of 5%.

IV. RESULTS

52% Where group I "(cases) ranged from 25-36 with a mean of 30.9 and sd ratio 3.37. And group II (control) ranged from 25-37 with a mean age of 31.51 and sd ratio 3.82.**Table (1)**

Table (1): Comparingamong the two studied groups in accordance to ages

	Cases (n = 30)	**Controls (n = 20)**	**t**	**P**
Age (years)				
Min. – Max.	25.0 – 36.0	25.0 – 37.0		
Mean ± SD.	30.90 ± 3.37	31.15 ± 3.82	0.244	0.808
Median (IQR)	30.50 (27.0 – 34.0)	31.0 (28.50 – 34.50)		

t: Student t-testing

p: p-value for comparisonamong the two groups

Women's MRI Endometrial Nodule show that 26(86.7%) were negative and 4(13.3%) were positive.

Table (2):Distribution of studied sample in accordance to patient's MRI Endometrial Nodule.

MRI Endometrial Nodule	**No.**	**%**
Negative	24	80.0
Positive	6	20.0
Total	30	100

Distribution of study group according to laparoscopic findings where stage-I:10%,stage-II: 13.3%, stage-III: 60%, and stage-IV:16.7%. **Table (3)**

Table (3):Distributing of the participated cases in accordance to staging by laparoscopic finding

Staging by laparoscopic finding	No.	%
Early		
Stage I	3	10.0
Stage II	4	13.3
Late		
Stage III	18	60.0
Stage IV	5	16.7

A significant changewas found among cases and controls in terms of methylation index of estrogen receptors where cases values being 0.02-132.9 with mean value12.99 and p value <0.05 and contol group being0.14-164.4 with a mean 66.04 and p-value>0.05. **Table (4)**

Table (4): Comparingamong the two groups regarding to methylation index

Methylation index	Cases (n = 30)	Controls (n = 20)	U	P
Min. – Max.	0.02–132.9	0.14– 746.4		
Mean ± SD.	12.99 ± 29.67	66.04 ± 164.4	159.000*	0.005*
Median (IQR)	1.30 (0.52–8.40)	17.80 (2.19–53.41)		

U: Mann Whitney testing

p: p-value for matchingamong the two groups

*: Statistical significance at p -value≤ 0.05

This table displays that there was no statisticallychangeamong methylation index and lapaoratory findings in both study and control group. **Table (5)**

Table (5): Correlation among methylation index and lab parameters in each group

Lab parameters	Methylation index	
	r_s	P

Cases		
Hemoglobin	–0.257	0.171
WBCs	0.084	0.659
Platelets	0.299	0.156
FBS	0.002	0.992
PTT	0.299	0.156
Control		
Hemoglobin	0.201	0.288
WBCs	–0.264	0.260
Platelets	0.253	0.282
FBS	0.258	0.272
PTT	0.080	0.675

r_s: Spearman coefficient **:**

There was statistical difference between methylation index of estrogen receptors and endometrial nodule found by ultrasound and magnetic resonance imaging where p value was <0.05. There was statistical differnce between methylation index and chocolate cyst found by ultrasound where p value<0.05. No statistical changewas found among methylation index and complain of pateints regarding pain and infertility where p value was >0.05.**Table (6)**

Table (6): Relation between methylation index and different in cases group (n=30)

	N	**Methylation index**			**U**	**P**
		Min. – Max.	**Mean ± SD.**	**Median**		
2D U/S chocolate cyst						
No	**2**	0.06– 0.26	0.16± 0.14	0.16	5.000	0.045
Yes	**28**	0.02–132.9	13.90±30.53	1.52		
2D U/S endometrial						

nodule						
No	**26**	0.02–132.9	14.92±31.49	1.75	17.000*	0.031*
Yes	**4**	0.06– 0.66	0.41± 0.29	0.45		
MRI endometrial nodule						
No	**26**	0.02–132.9	14.92±31.49	1.75	17.000*	0.031*
Yes	**4**	0.06– 0.66	0.41± 0.29	0.45		
Pain						
No	**9**	0.02–65.07	12.85±20.36	8.40	66.000	0.209
Yes	**21**	0.06–132.9	13.04±33.32	1.12		
Infertility						
No	**15**	0.06–132.9	12.84±33.94	1.23	112.000	1.000
Yes	**15**	0.02–85.26	13.13±25.90	1.68		

U: Mann Whitney testing

p: p-value for comparisonamongthe groups

*: Statistical significance at p-value ≤ 0.05

Table (7):Relation between methylation index and staging by laparoscopic finding in cases group (n=30)

Staging by laparoscopic finding	**N**	**Methylation index**			**H**	**P**
		Min. – Max.	**Mean ± SD.**	**Median**		
Early						
Stage-I	**3**	0.21 – 1.83	0.85 ± 0.87	0.49		
Stage-II	**4**	0.06 – 0.80	0.30 ± 0.35	0.16	8.663*	0.034*
Late						
Stage-III	**18**	0.02 – 132.87	20.02 ± 36.91	20.56		

Stage-IV	**5**	1.37 – 10.44	5.09 ± 4.11	3.56		

H: H for **Kruskal Wallis testing**

p: p-value for comparison among the groups

*: Statistical significance at p-value ≤ 0.05

V. DISCUSSION

ERα seems to be the principalintermediary of estradiol-persuadedprogesterone (P4) actions in these tissues, and P4 operates its roles in the endometrium by bonding to the nuclear receptors PgRA and PgRB. Epigenetic mechanism moderates the dynamical regulations of the estrogen receptor genes and their roles.**(Rody et al., 2005)**

This work aiming to evaluate the methylation condition of estrogen receptors gene promotor in cases with endometriosis. The ultimate objective is to investigate its role as a potential diagnostic marker in the disease process.

A cross-sectional study was performed on fifty casesemployed from gynecology clinic in Shatby Maternity Hospital, Alexandria University complaining of pain or infertility. The duration of the study ranged from 6-12 months.

Women's age show that more than half of our patients their age less than 30 years 16(53.3%); patients age was ranged between 25-36 years with mean± S.D. 30.90±3.397 years.

Women's MRI endometrial nodule showed that 26 patients (86.7%) were negative and 4 patients (13.3%) were positive.

In the study in our hands, our major finding was endometriotic tissue have lower methylation indeices in comparison to the eutopic endometrium.so there is hypomethylation in estrogen receptor genes in cases of endometriosis in comparison to control group.

We also found that there was statistically significance in laparoscopy findings with cases of endometriotic nodules and hypometylation of estrogen receptors.

The other laparoscopic finding like ovarian endometrioma and tubal adhesions, there was no statistical significance.

However, we could not prove sufficient evidence for hypomethylation of estrogen receptors in cases of ovarian endometrioma or tubal adhesions.

Our findings were in accordance with the results by**Meyer et al.,(2014)**as they reported that fresh endometriotic tissues were gathered from 44 premenopausal cases (mean age, 35.1±6.8 yrs.) who diagnosed by laparoscopic and operative resections (nodule or segmented resection of the rectum).

Meyer et al.,(2014) revealed that promoter area B of the PGR gene varied in respects to the methylation condition amongeutopic endometrium and deep endometriosis conciliatory the rectum: methylated alleles were preciselydiagonosed in the endometrioticlesions, whereas endometrium specimensexhibited only un-methylated alleles. These resultspropose that the epigenetic changemight be a viable endometriosis bio-marker.

Maekawaet al.,**(2017)** found that ovarian endomrtrioma had aberrant DNA methylation in the T-DMRs of ESR1 and that the DNA methylation is accompanying with the reduced expressions of ESR1 in ovarian endometrioma.

In the study of **Zidan et al., (2015)**nonsignificantchange was foundamong the studied groups regarding to the cycle phase (The studied patients were divided into 60-females (mean aging 32±6.3 yrs.), experiencingsurgical laparoscopic for endometriosis and healthy endometrial specimens were gathered from 30 fertile females as control-group (mean aging 33±5.2 yrs.). They didn't have endometriosis but haveexperiencedsurgical operationfor instance tubal ligation, in vitro fertilizations, hysterectomy for cervical dysplasia or curettement for cervical erosion).

We found one study in literature conducted by **Xue et al., (2007)** has assessed the methylatingcondition of the ESR2 gene in 8 ovarian endometriomas by bisulfite-adapted DNA sequencing. The researchersdefinedelevated values of ESR2 transcriptionsaccompanying with hypomethylation of the promoter area compared to endometrium.

By means of the same MS-PCR primer group, **Wu et al., (2008)**concluded the existence of fractional methylation in this promoter in the epithelial constituent of peritoneal and ovarian endometriotic implants, but not in the promoter area that control the expressions of the A isoform of P4 receptors.

Furthermore, the researchersconcludedsmall levels of PGRB expressions in endometrioticepithelial cells. Their consequencesproposed that P4 resisting in endometriosis generally and the down-regulating of PR-B, but not PR-A, in specific, are a consequence of promoter hypermethylation of PR-B, but not PR-A.

Yamagata et al., (2014) compared methylation shapes of the eutopicendometrium from females with/without endometriosis and ovarian endometrial cysts, some genes were unlikely methylated in the endometrium, whileadditionalhyper-methylated and hypo-methylated CpGs were found in the endometria ovarian cysts.

Dyson et al revealed that, **(2014)** in 42,248 unlikely methylated CpGs that were studied, 403-genes established significant dissimilar methylation forms. A dis-proportionally larger number of transcriptions factors had varied methylation profiles and numerous of these genes are previouslyrecognized to be participating in the procedure of decidualization and the pathophysiology of endometriosis.

Geneticists have listed the subsequentinfluences for the failing of applicant gene investigations to ascertain the genetic base of complicatedinflammation diseases like, endometriosis. (i) The basic biologically hypothesis might be invalid. (ii) These reportscharacteristically investigated a restricted number of genes in a possiblysignificant biological path-way. (iii) A partial number of variations in a gene are analyzed. (iv) patients (endometriosis) and control-group (endometriosis-free) mightnot be precisely outlined. (v) Sample sizes might not be adequate to recognized the influence sizes that are predictable for variants affecting a compound trait(13)

The discrepancy between the different reported studies may be attributed to various factors as the heterogeneity of the study population, the inclusion and exclusion criteria, the starting point and the ending point of the observation period.

Other mechanisms like the bonding of transcriptions factors might be accompanying with the differentially expression of estrogen receptors amongeutopic endometrium and endometriotic tissue.

VI. CONCLUSION

The finding suggests that, Estrogen receptor gene hypomethylation in endometriotic tissues. Progesterone resistance is found in endometriosis generally with down regulating of PR-B, as a result of promoter hypermethylation of PR-B.

REFERENCES

1. **Ballestar E. (2011):** An introduction to epigenetics. Adv Exp Med Biol; 711:1-11.
2. **Dyson MT, Roqueiro D, Monsivais D, Ercan CM, Pavone ME, Brooks DC, et al. (2014):** Genome-wide DNA methylation analysis predicts an epigenetic switch for GATA factor expression in endometriosis. PLoS Genet; 10(3):e1004158.
3. **Grandien K, Berkenstam A, Gustafsson JA. (1997):** The estrogen receptor gene: promoter organization and expression. Int J Biochem Cell Biol; 29(12):1343-69.
4. **Hirata S, Shoda T, Kato J, Hoshi K. (2001):** The multiple untranslated first exons system of the human estrogen receptor beta (ER beta) gene. J Steroid Biochem Mol Biol; 78(1):33-40.
5. **Hummelshoj L, Prentice A, Groothuis P. (2006):** Update on endometriosis. Womens Health; 2(1):53-6.
6. **Illingworth RS, Bird AP. (2009):** CpG islands--'a rough guide'. FEBS Lett; 583(11):1713-20.
7. **Maekawa R, Taketani T, Mihara Y, Sato S, Okada M, Tamura I, et al. (2017):** Thin endometrium transcriptome analysis reveals a potential mechanism of implantation failure. Reprod Med Biol; 16(2):206-27.
8. **Meyer JL, Zimbardi D, Podgaec S, Amorim RL, Abrão MS, Rainho CA. (2014):** DNA methylation patterns of steroid receptor genes ESR1, ESR2 and PGR in deep endometriosis compromising the rectum. Int J Mol Med; 33(4):897-904.
9. **Rody A, Holtrich U, Solbach C, Kourtis K, von Minckwitz G, Engels K, et al. (2005):** Methylation of estrogen receptor beta promoter correlates with loss of ER-beta expression in mammary carcinoma and is an early indication marker in premalignant lesions. Endocr Relat Cancer; 12(4):903-16.
10. **Vincent K, Kennedy S, Stratton P. (2010):**Pain scoring in endometriosis: entry criteria and outcome measures for clinical trials. Report from the Art and Science of Endometriosis meeting. Fertil Steril; 93(1):62-7.

11. **Wang D, Chen Q, Zhang C, Ren F, Li T. (2012):** DNA hypomethylation of the COX-2 gene promoter is associated with up-regulation of its mRNA expression in eutopic endometrium of endometriosis. Eur J Med Res; 17(1):12.

12. **Wu Y, Shi X, Guo SW. (2008):** The knockdown of progesterone receptor isoform B (PR-B) promotes proliferation in immortalized endometrial stromal cells. Fertil Steril; 90(4):1320-3.

13. **Xue Q, Lin Z, Cheng YH, Huang CC, Marsh E, Yin P, et al. (2007):** Promoter methylation regulates estrogen receptor 2 in human endometrium and endometriosis. Biol Reprod; 77(4):681-7.

14. **Yamagata Y, Nishino K, Takaki E, Sato S, Maekawa R, Nakai A, et al. (2014):** Genome-wide DNA methylation profiling in cultured eutopic and ectopic endometrial stromal cells. PLoS One; 9(1):e83612.

15. **Zidan HE, Rezk NA, Alnemr AA, Abd El Ghany AM. (2015):** COX-2 gene promoter DNA methylation status in eutopic and ectopic endometrium of Egyptian women with endometriosis. J Reprod Immunol; 112:63-7.