Journal of Clinical Medicine: Current Research **ISSN: 2832-7551**
Volume 3 (2023) 10 Pages
DOI: http://dx.doi.org/10.53043/2832-7551.JCMCR.3.008

Research Article* | *Open Access

Exploring the Diagnostic Potential of IHC and Next-Generation Sequencing Approaches for Endometrial Carcinoma Detection and Classification

Azhin Saber ALI[1,2]* and Lana Sardar ALALEM[2]

[1]Department of Medical Laboratory Technology, Shaqlawa Technical College, Erbil Polytechnic University, Erbil, Iraq
[2]Department of Biology, College of Science, Salahaddin University-Erbil, Erbil, Kurdistan region, Iraq

***Correspondence:** Azhin Saber ALI, Department of Medical Laboratory Technology, Shaqlawa Technical College, Erbil Polytechnic University, Erbil, Iraq

Received: June 20, 2023
Accepted: June 27, 2023
Published: July 03, 2023

Citation: Azhin Saber ALI, Lana Sardar ALALEM. Exploring the Diagnostic Potential of IHC and Next-Generation Sequencing Approaches for Endometrial Carcinoma Detection and Classification. J Clin Med Current Res. (2023);3(2): 1-10

Key words: Endometrioid carcinoma, Microsatellite instability, PTEN and E-cadherin

1. Abstract

Background: Globally, in 2018 endometrial cancer (EC) regarded as the sixth most commonly diagnosed cancer and the fourteenth leading cause of cancer with more deaths in females. Nowadays classical and modern classifications can be performing for diagnosing various types of cancers including endometrial cancer. Based on this our **Objective of study** is to determine the effectiveness of immunohistochemical observation, a quick and easy method for determining MSI and/or other types of EC which based on modern classification. As molecular study takes more cost we want to compare the both methods and showing the possibility of doing IHC instead of molecular study.

Methods: This study designed to establish a small gene panel of Next Generation Sequencing NGS of endometrial cancer patients targeting 12 genes; MSH6, MLH3, MSH3, CDH1, PTEN, MSH2, MLH1, PMS2, FGFR2, BRCA1, MUTYH and AREL1. By using DNBSEQ-G400 Platform, Human Core Exome kit and Python software for analysis is used. At the same time, Dako kit used to performing IHC for six primary antibodies used to detect each of MSH6, MSH2, MLH, PMS2, PTEN and CDH1. The primary antibodies were applied on 5 µm formalin- fixed paraffin- embedded (FFPE).

Results; histopathological examinations showed that all patients were at stage II endometrioid endometrial carcinoma. The FIGO classification were Ia and Ib. Microsatellite instability (MSI), nuclear staining immunoreaction for PTEN antibodies and membranous E- cadherin immunoreactions observed through IHC study. The molecular studies detected several polymorphisms which have clinical significant and some of them have conflict interpretations.

Conclusions; we considered that, simply immunoreaction staining procedure can be use as alternative method for MSI phenotype detection rather than any type of more expensive and complex method of NGS. Concerning PTEN and ECAD1 antibodies reactions, also could be dependable in diagnosis and treatment and performing them if molecular diagnosis not available.

2. Introduction

Globally, in 2018 endometrial cancer (EC) regarded as the sixth most commonly diagnosed cancer and the fourteenth leading cause of cancer with more deaths in females [1]. The annual incidence is estimated at 10.8 per 100,000 women in the world. The incidence of this cancer is four times more high in the industrialized countries of Europe and America North compared to Asia (including Japan), Africa and South America [1]. There is no precise data for endometrial cancer in Iraq country, however some evidences showing elevating uterine cancer in general including endometrial cancer in some cities [2].

More than 30 years ago, based on hormonal and clinical characters Bookhman classified EC into two types, type I EC and type II EC [3]. Type I EC are estrogen-dependent, mainly low grade, hormone-receptor-positive adenocarcinomas with endometrioid morphology and are often referred as endometrioid endometrial cancers and account for approximately 85% of all EC usually diagnosed at an early stage and characterized by a good prognostic. Type II EC are characterized by non-endometrioid subtypes such as serous, clear-cell and undifferentiated carcinomas. They generally are high- grade, hormone-receptor negative, and have poor prognosis [3]. According to the International Federation of Gynecology and Obstetrics (FIGO) adopted a surgical pathologic staging since 1988 and in 2009, FIGO updated the staging system classified patients into prognostic groups based on extent of disease [4,5].

New classification is based on molecular features, according to The Cancer Genome Atlas (TCGA), establish a new molecular classification of EC by identifying 4 distinct classes of tumor listed from best to worst prognosis [6]. Efforts have been made to classify ECs into these 4 molecular subgroups using techniques available in routine [7,8,9]. Which include, the POLE (DNA polymerase ε) ultramutated group, the hypermutated/microsatellite unstable (MSI) group, the copy number low/microsatellite stable group and the copy number high (serous-like) group.

2.1 Objectives of study

The objectives of this study were to determine the effectiveness of immunohistochemical observation, a quick and easy method for determining MSI and/or other types of EC which based on modern classification. As molecular study takes more cost we want to compare the both methods and showing the possibility of doing IHC instead of molecular study.

3. Methodology

3.1 Study design

Sample collection: Samples collected at Erbil maternal hospital from those patients who previously diagnosed by having endometrial carcinoma through their curettage biopsy examination, then gynecologists did total abdominal hysterectomy TOH for them. We took a small piece of tumor region (the size of the tissue from each EC patient was ≥0.5 × 0.5 × 0.5 cm3) immediately after removing their uteri, putting in cold PBS for transporting to lab for molecular study. Routinely, the whole removed organs put in buffered Formalin for histopathological examination.

Cancer Gene Panel: Genomic DNA extracted from the fresh tissue by using geneaid kit. Then we established a small cancer gene panel, covering 12 genes for next-generation sequencing (whole exome sequencing=WES); MSH6, MLH3, MSH3, CDH1, PTEN, MSH2, MLH1, PMS2, FGFR2, BRCA1, MUTYH and AREL1. By using DNBSEQ-G400 Platform and Human Core Exome kit and sequencing was performed by DNA Laboratuvarları Genetik Hastalıklar Tanı Merkezi. Python software for analysis is used (Figure 1).

Histopathological Examination: Routine histopathological examination was performed for our samples through putting them in 10% formal saline for one week for fixing the tissue, then underwent serial treatment including dehydration, clearing, infiltration, embedding, sectioning and finally staining with hematoxylin and eosin.

Immunohistochemistry detection: Six primary antibodies used to detect each of MSH6, MSH2, MLH, PMS2, PTEN and CDH1 by using Dako kits to detect some of mutated gene immunohistochemically. According to the manufacturer's instruction. The primary antibodies were applied on 5 μm formalin- fixed paraffin- embedded (FFPE).

Immunohistochemical interpretations: The six slides from each tumor block staining for MLH1, MSH2, MSH6, PMS2, PTEN and CDH1 were assessed. Semiquantitative scoring system a was used to minimize interobserver variation [10,11,12]. A valid result required the existence of internal control immunopositivity, the lymphocytes, endometrial stromal cells, and the epithelial cells of the nearby normal mucosa are the internal controls. On a scale from 0 to 3, the level of immunoreactivity in the malignant epithelial cells' nuclear compartment was assessed. This rating system was based on a comparison of the tumor cells' level of reactivity to the positive control cells. A score of 0 meant there was no reactivity, while a score of 3 meant there was reactivity in the

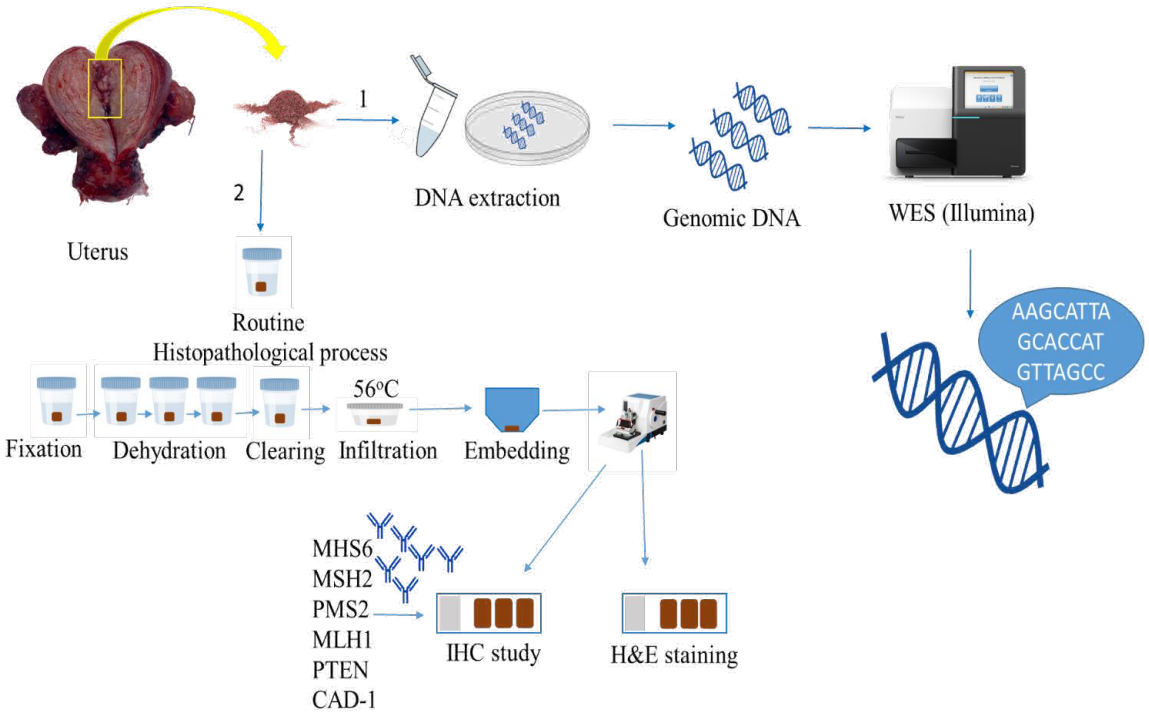

Figure 1: An illustration diagram for methodology. WES: Whole Exome Sequencing, H&Estaining: Hematoxylin and Eosin staining, IHC study: Immunohistochemistry study.

tumor cells that was comparable to that of positive control cells. For the percentage positivity which means distribution of the genes within the tumor cells, the scoring was as follows; no tumor cell immunopositivity equal to 0 score, 1-10% positive tumor cells equals to score 1, 11- 50% positive tumor cells equals to score 2, 51-80% positive tumor cells equals to score 3 and more than 80% is score 4 [10,12]. For making one number for the semiquantitative scores the intensity and percentage of immunopositivity for each antibody were multiplied to produce a number between 0 to 12 [11].

4. Results

4.1 Whole exome sequencing WES results

The observed polymorphism in table 1 analyzed as clinical significance, from the current variations most of them found in dbSNP but not found as a clinical significance for EC in Clin Var database. About 13 of them not found even in dbSNP, such as PTEN (89720633->T) for the first patient (EC1), see remaining 12 variations in table 1, these are named novel variations.

Within these polymorphisms, some of them located at intron regions and others located within exon region. The former included deletions, insertions and deletion/insertion, while the later has many types of mutations which means functional consequences including synonymous single nucleotide variation (SNV), and non-synonymous SNV. Within non- synonymous SNV most of them were missense variants (MLH3 for EC2, MLH1, PMS2 and BRCA1 for EC3 and MSH2 for EC4) with only one non frameshift insertion (MSH3 for EC2) (Table 1).

Table 2 showing the variation which have conflicting interpretations. conflicting interpretations come define as genetic results from multiplex panel testing utilized in clinical practice are frequently interpreted differently, which may have an impact on how a patient is managed [13,14]. Some variations in MSH2 for EC1, EC2 and EC4 and MSH6 for EC2 were found in Clin Var database as conflicting interpretations. Three of them found in dbSNP database but no data recorded for EC as clinical significant. And one of them (MSH2) was novel as not found in dbSNP database.

4.2 Routine histopathological examination

The architecture of endometrial layer of patients with endometrioid carcinoma characterized by the tubular, cribriform (a malignant epithelial growth that takes the shape of massive nests pierced by numerous, very spherical gaps of various sizes). The tumor cells are columnar, stratified and showed different cytonuclear atypia. Our results also

Table 1: Nucleotide polymorphism identified in EC patients.

Patients	Gene	Variant coordinate	AA change	Zygosity	Mutation type	External database\ clinical significant
EC1	MSH6	NC_000002.11:g.48025764C>T	p.Y214Y	het	synonymous SNV	dbSNP/ not reported in Clin Var for EC
		NC_000002.11:g.48032572C>T		het	intron	dbSNP/ not reported in Clin Var for EC
	PTEN	89720633->T		het	intron	Novel
	MSH2	NM_000251.3	c.942+24_ 942+29del	het	intron	dbSNP/ not reported in ClinVar for EC
		NC_000002.11:g.476 90162G>T		het	intron	dbSNP/ not reported in Clin Var for EC
		NC_000002.11:g.476 98179A>G	p.K>K	het	synonymo us SNV	dbSNP/ not reported in Clin Var for EC
	MLH1	NC_000003.11:g.370 67097A>T		het	intron	dbSNP/ not reported in Clin Var for EC
	PMS2	NM_000535.7	c.706-4del	het	intron	dbSNP/ not reported in Clin Var for EC
		NC_000007.13:g.602 2626C>T		het	intron	dbSNP/ not reported in Clin Var for EC
	BRCA 1	NM_007294.4	c.441+64d el	hom	intron	dbSNP/ not reported in Clin Var for EC
	MUT YH	NC_000001.10:g.458 00156C>T		het	synonymo us SNV	dbSNP/ not reported in Clin Var for EC
EC2	MSH6	NC_000002.11:g.480 26172C>T	p.A>A	het	synonymo us SNV	dbSNP/ not reported in Clin Var for EC
	MLH3	NC_000014.8:g.7551 3463A>G	p. S>S	het	missense	dbSNP/ not reported in Clin Var for EC
	MSH3	5:79950724-> CCGCAGCGC	p.P63_P6 4insAAP p.A19_A2 0insGAA	het	Non frameshift insertion	Novel
	CDH1	NC_000016.9:g.6884 2480G>C	Intron	het	SNV	dbSNP/ not reported in Clin Var for EC
		16: 68857544->A	Intron	het	DELINS	Novel
		NC_000016.9:g.6886 2165C>T	p. N>N	het	synonymo us SNV	dbSNP/ not reported in Clin Var for EC
	PTEN	10: 89720634T>-	Intron	het	Del	Novel
	MSH2	NC_000002.11:g.477 02191A>G	p.N>S	het	nonsynon ymous SNV	dbSNP/ not reported in Clin Var for EC
		2: 47641560AAAA>-	Intron	het	DEL	Novel
	MLH1	NC_000003.11:g.37053549C>T	p.T>T	het	synonymous SNV	dbSNP/ not reported in Clin Var for EC
		3: 37067094 TATATATT>-	intron	het	Del	Novel
	PMS2	7: 6037058A>-	intron	het	Del	Novel
	BRCA1	17: 41256075A>-	Intron	het	Del	Novel
	MUTYH	NC_000001.10:g.45800033T>C	Intron	het	SNV	dbSNP/ not reported in ClinVar for EC
EC3	MSH6	NC_000002.11:g.48032754A>T	Intron	het	SNV	dbSNP/ not reported in Clin Var for EC
	MSH2	2: 47641560AAA>-	Intron	het	DEL	Novel
		NC_000002.11:g.47694037T>C	Intron	het	SNV	dbSNP/ not reported in Clin Var for EC
	MLH1	3: 37067094 TATATATTT>-	intron	het	DEL	Novel
		NC_000003.11:g.37067306G>A	p. S>N	het	missense	dbSNP/ not reported in Clin Var for EC

Continued...

EC3	PMS2	7: 6037058AA>-	Intron	het	DEl	Novel
		NC_000007.13:g.602 6384C>T	Intron	het	SNV	dbSNP/ reported in Clin Var for EC
		NC_000007.13:g.6026942G>T	p. T>K	het	missense	dbSNP/ not reported in ClinVar for EC
		NC_000007.13:g.6043386G>A	p. A>A	het	synonymous SNV	dbSNP/ not reported in Clin Var for EC
	BRCA1	17: 41256075A>-	Intron	het	DEL	Novel
		NC_000017.10:g.41201364T>C	Intron	het	SNV	dbSNP/ not reported in ClinVar for EC
		NC_000017.10:g.41216206T>C	Intron	het	SNV	dbSNP/ not reported in Clin Var for EC
		NC_000017.10:g.412 45471C>T,	p. D>N	het	missense	dbSNP/ not reported in Clin Var for EC
EC4	MSH6	NC_000002.11:g.480 32717T>A	Intron	het	SNV	dbSNP/ not reported in Clin Var for EC
		NC_000002.11:g.48 010558C>A,	p.R62R	het	synonymo us SNV	dbSNP/ not reported in Clin Var for EC
	CDH1	16: 68857544->A	Intron	het	INS	Novel
	MSH2	2: 47641560AAAAA>-	intron	het	DEL	Novel
		NC_000002.11:g.476 43457G>A	p.G>D	het	missense	dbSNP/ not reported in Clin Var for EC
		NC_000002.11:g.476 94037T>C	Intron	het	SNV	dbSNP/ not reported in Clin Var for EC
	MLH1	3: 37067094 TATATATTT>-	Intron	het	DEL	Novel
		NC_000003.11:g.370 67097A>T	Intron	het	SNV	dbSNP/ not reported in Clin Var for EC
	PMS2	7: 6037058A>-	Intron	het	DEL	Novel
		NC_000007.13:g.604 3495T>C	Intron	het	SNV	dbSNP/ not reported in Clin Var for EC

Table 2: Nucleotide polymorphism with conflicting interpretation clinical significance.

Pati ents	Ge ne	Variant coordinate	AA change	Zyg osity	Mutation type	CLN SIG	External database\ clinical significant
EC1	MS H2	NM_000251.3	c.942+24_ 942+29del	het	intron	Conflicting	dbSNP/ not reported in Clin Var for EC
EC2	MS H6	NC_000002.11:g. 48026172C>T	p.A>A	het	synonymou s SNV	Confl icting	dbSNP/ not reported in Clin Var for EC
	MS H2	NC_000002.11:g. 47702191A>G	p.N>S	het	nonsynonymous SNV	Conflicting	dbSNP/ not reported in Clin Var for EC
		2: 47641560AAAA>-	Intron	het	DEL	Confl cting	Novel
EC4	MS H2	2: 47641560AAAA A>-	intron	het	DEL	Confl cting	Novel

revealed by [15]. Cytonuclear atypia which are a precursor for endometrioid carcinoma characterized by loss of polarity, rounded nuclei, anisokaryosis, hyper or hypochromasia and a more eosinophilic cytoplasm [8,16] (Figure 2).

Table 3 showing the FIGO classifications for all patients separately. Each of EC1 and EC2 are in Ib FIGO stage, pT1a, pN0 in TNM staging. pT1a means only affects the endometrium or only penetrates around half of the myometrium. pN0 means Only small a number of cancer cells less than 0.2 mm in diameter (which are isolated tumor cells) or none at all in any adjacent nodes are seen, also showed by [17]. While each of EC3, EC4 and EC6 are of the same FIGO staging which are T1b, Nx, Mx, Ib. T1b signifies that the cancer is between 1-2 cm in size. Both Nx and Mx means nothing has been identified for nearby lymph nodes and unable for mearing metastasis, respectively. Finally,

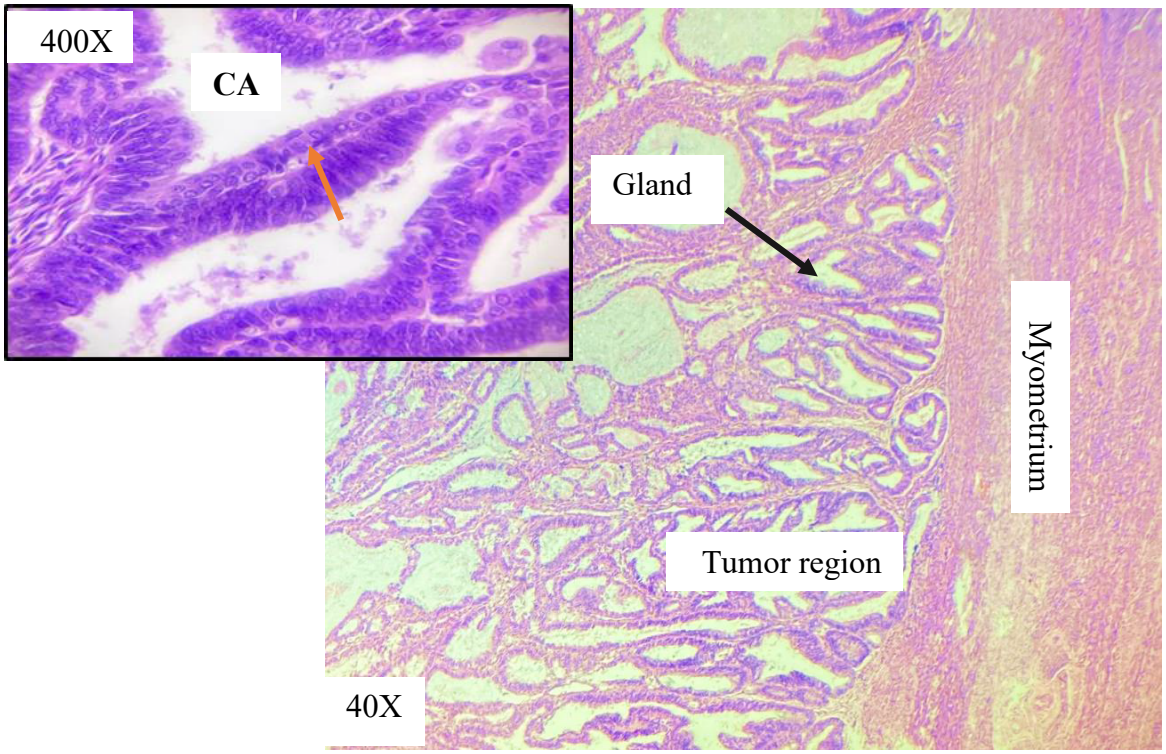

Figure 2: Endometrioid endometrial carcinoma, tumor region showing tubular differentiation of gland (black arrow) and cells are columnar, stratified and showed different cytonuclear atypia (CA) (green arrow).

Table 3: Clinical and pathologic data of patients.

		EC1	EC2	EC3	EC4	EC5	EC6
Age		**51**	**56**	**70**	**68**	**56**	**55**
Grade		**2**	**2**	**2**	**2**	**2**	**2**
FIGO staging	**T**	**pT1a**	**pT1a**	**T1b**	**T1b**	**T1a**	**T1b**
	N	**pN0**	**pN0**	**N0**	**Nx**	**Nx**	**Nx**
	M	**Mx**	**Mx**	**Mx**	**Mx**	**Mx**	**Mx**
	stage	**Ia**	**Ia**	**Ib**	**Ib**	**Ia**	**Ib**
Myometrium invasion		**half**	**half**	**half**	**half**	**half**	**half**

EC5 was at T1a, Nx, Mx clearly means this patient had endometrioid carcinoma which only affects the endometrium or only penetrates around half of the myometrium (T1a), in addition to no information about adjacent lymph nodes and metastasis, Nx and Mx respectively.

Immunoreactions observation and interpretations: The reactions for four MMR antibodies showed in Figure 3. These biomarkers had different level of reaction intensities and had different regional reactions. For all patients the nuclear staining for both MSH6 and MSH2 observed in tumor regions while nuclear staining reactions of both MLH1 and PMS2 occurred at non-tumor regions.

Nuclear staining of PTEN antibodies reactions occurred in normal region and punched out in cancerous glandular region. While membranous E-CAD1 antibodies reactions observed in glandular tumor regions. The two later biomarker reactions showed in Figure 4.

The nuclear staining observed for all MMR proteins. IHC study for these genes PMS2 and MLH1 expressed in non- tumor region while MSH6 and MSH2 expressed in tumor region. PTEN also is have nuclear staining characteristics but observed at non-tumor region. And membranous antibodies reaction seen for ECAD1 under microscopic examinations.

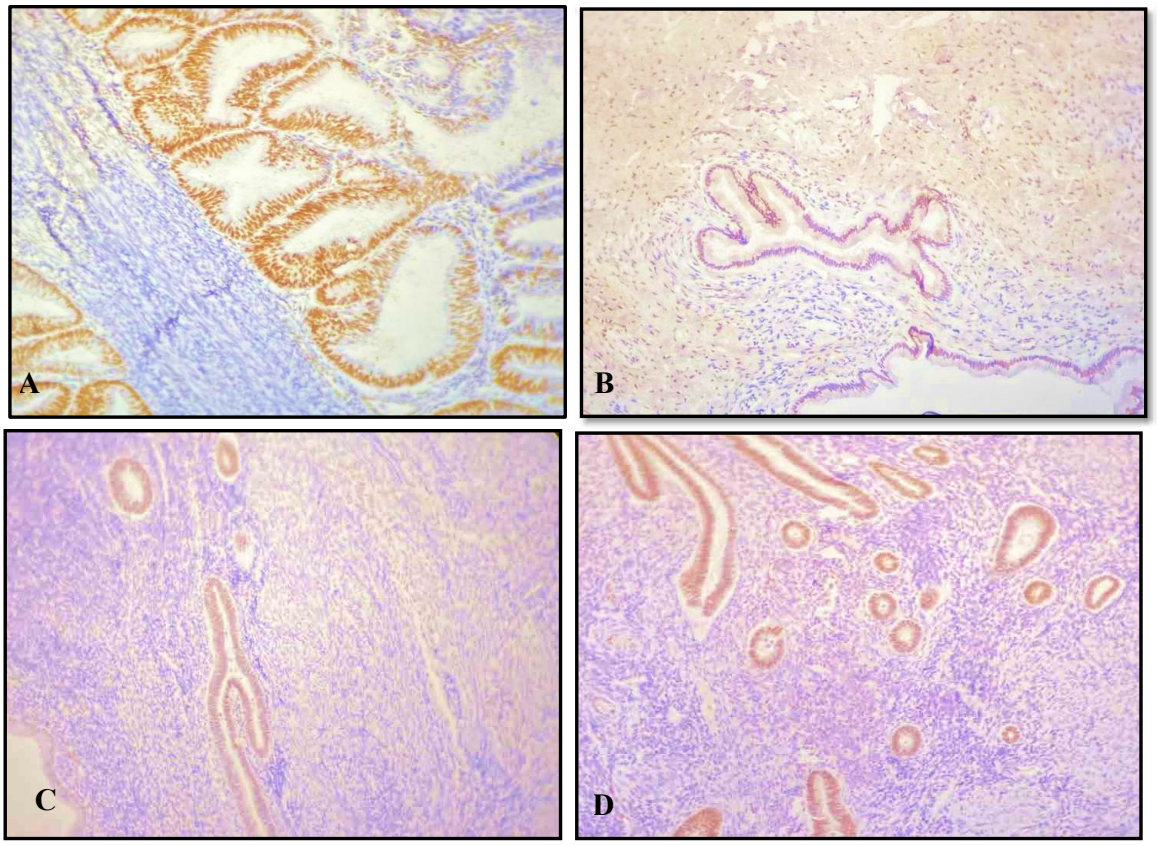

Figure 3: Nuclear immunoreaction of MMR proteins in EC patients. A: MSH6 and B: MSH2 antibodies are detected in tumor regions while C; MLH1 and D: PMS2 antibodies are detected innon-tumor regions. 100X.

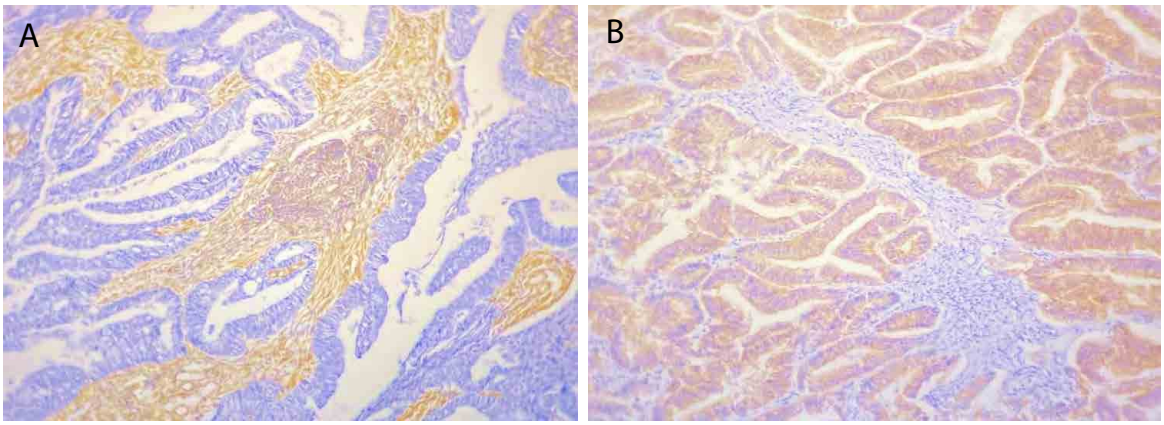

Figure 4: Nuclear immunoreaction of PTEN antibodies, in non-tumor regions (A) andmembranous immunoreaction of E-CAD1 antibodies (B). 100X.

The staining intensity score and percentage positivity score were showed in table 3. The scoring system for 6 antibodies were at different level for each samples. There was no MLH1 antibodies reaction for the EC5 and no PMS2 antibodies reaction for the EC1 even after repetition of procedure. The highest score was MSH6 antibodies for EC6 (Table 4).

Table 4: IHC assessment (staining intensity score · percentage positivity score).

Patients	MSH6	MSH2	MLH1 non- tumor region	PMS2 non- tumor region	PTEN non- tumor region	ECAD1
EC1	6	1	1	0	2	6
EC2	8	3	1	3	8	4
EC3	4	3	4	6	3	4
EC4	2	2	2	4	6	8
EC5	9	9	0	2	3	1
EC6	12	1	3	3	3	8

5. Discussion

The result of histopathological examinations of all patients revealed that the cancer type may belonging to type I EC according Bokhman, 1983 which estrogen dependent [3].

WES is an alternative to whole genome sequencing (WGS) because the exome makes up only 2% of the human genome yet contains 85% of known disease-related mutations [18,19]. WES provides a number of advantages over WGS, including lower cost, quicker data analysis, and simpler data management [20]. Considering the results of WES for 4 patients we found many variations and showed in two tables separately, table 1 including the polymorphisms which are interpreted as clinical significant, table 2; variation that have different interpretations known to be conflicting.

Microsatellites are DNA elements composed of short repetitive motifs that are prone to misalignment and frameshift mutations during cell division. In healthy cells, the ensuing small indels or single-base mispairs are corrected by heterodimer enzyme complexes of the DNA mismatch repair (MMR) system encoded by the key MMR genes *MLH1*, *MSH2*, *PMS2* and *MSH6* [21,22]. DNA mismatch repair deficiency (dMMR) results in the progressive accumulation of genetic mutations with each cell replication, potentially dysregulating many oncogenes or tumor suppressor genes. The molecular hallmark of dMMR is MSI (microsatellite instability), with expansions or contractions in the number of tandem repeats throughout the genome. This phenomenon is observed in a considerable proportion of colorectal, endometrial, gastric, pancreatic, brain, biliary tract, urinary tract and ovarian tumors [21,23,22]. A MSI or dMMR was defined as the lack of at least one MMR protein [12].

The heterodimer arrangements are presented for MMR proteins as the four proteins are locating in sequence of two together as follows; MLH1 with PMS2 and MSH2 with MSH6 [24]. This results also detected in our IHC study for these genes as PMS2 and MLH1 are expressed in non- tumor region while MSH6 and MSH2 expressed in tumor region. Relay on their functional structure, it is feasible to carry out an immunohistochemical panel of PMS2 and MSH6 antibodies as early screening for MMR deficiency [24]. Based on some previous studies for immunohistochemical evaluation we consider the presence or absence of nuclear staining, assuming that a positive reaction of tumor cells is considered intact protein expression (MSS phenotype) and that lack of expression, with positive internal control, is regarded MSI phenotype [25-28].

Nuclear staining observation of PTEN showed in Figure 4. Pten normally present in the nucleus, cytoplasm, and cell membrane [29] which can appear weak and somewhat variable. True Pten loss is characterized by complete absence of nuclear and cytoplasmic expression in glands (excluding intraglandular leukocytes, which can be abundant) [30]. In normal endometria, Pten loss in scattered glands was a common occurrence, in accordance with previous landmark studies [31,32,33,32].

One of the distinguishing features of a malignant tumor is altered cell adhesion, which includes variations in the expression and distribution of adhesion molecules [34-36]. Numerous cancers of various epithelial origins have been found to express E-cadherin less frequently, a protein necessary for the formation of cell-cell interactions [37]. This cell to cell contact define as adherent junctions [38].

Homogenous distribution of E- cadherin found through immunoreactivity test for our sample in glandular epithelium, figure 4.3. Non-nuclear region for this gene expression is presented by previous studies [36]. Depending on Pećina-Slaus, 2003 evidence who revealed that low expression of E-cadherin is considering as one of the primary molecular mechanisms essential in malfunction of the cell-cell adhesion system, leading to cancer invasion and metastasis [39], and linking this observation with our results for E-cadherin, could explain that the intense interaction of this antibody may since of low grade of cancer with no metastasis.

6. Conclusions

Regarding our evaluation system in dMMR proteins determination through IHC study, which has already been utilized to determine the MSI phenotype [26,28,40,41], we considered that, simply immunoreaction staining procedure can be use as alternative method for MSI phenotype detection rather than any type of more expensive and complex method of NGS.

Considering PTEN and ECAD1 antibodies reactions, also could be dependable in diagnosis and treatment and performing them if molecular diagnosis not available.

7. Recommendations

More studies required by evaluating larger samples size at different stage of endometrial cancer.

7.1 Authorship contribution statement

Both authors made contributions to the planning and design of the study. Azhin Saber Ali and Lana Sardar Alalem prepared the material and collected and analyzed the data. Azhin Saber Ali wrote the first draft of the manuscript, and both authors provided feedback on earlier drafts. The final manuscript was read and approved by both writers.

7.2 Ethical approval

It was obtained from the Salahaddin university College of science no: 4S/505.

7.3 Funding

There has been no specific funding awarded for this research.

12. References

1. BRAY, F., FERLAY, J., SOERJOMATARAM, I., SIEGEL, R. L., TORRE, L. A., *et al.*Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018, 68, 394-424https:// DOI: 10.3322/caac.21492.
2. K, M. A., ABDULLAH, O. S., AMIN, A. M. S., MOHAMED, Z. A., HASAN, B., *et al.*Cancer Incidence in the Kurdistan Region of Iraq: Results of a Seven-Year Cancer Registration in Erbil and Duhok Governorates. *Asian Pac J Cancer Prev* 2022, 23, 601-615https:// DOI: 10.31557/apjcp.2022.23.2.601.
3. BOKHMAN, J. V.Two pathogenetic types of endometrial carcinoma. *Gynecol Oncol* 1983, 15, 10-7https:// DOI: 10.1016/0090-8258(83)90111-7.
4. CREASMAN, W.Revised FIGO staging for carcinoma of the endometrium. *Int J Gynaecol Obstet* 2009, 105, 109https:// DOI:10.1016/j.ijgo.2009.02.010.
5. PECORELLI, S.Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. *Int J Gynaecol Obstet* 2009, 105, 103-4https:// DOI: 10.1016/j.ijgo.2009.02.012.
6. NETWORK, C. G. A. R.Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, Robertson AG, Pashtan I, Shen R, et al. Integrated genomic characterization of endometrial carcinoma. *Nature* 2013, 497, 67-73.
7. STELLOO, E., BOSSE, T., NOUT, R. A., MACKAY, H. J., CHURCH, D. N., *et al.*Refining prognosis and identifying targetable pathways for high-risk endometrial cancer; a TransPORTEC initiative. *Modern pathology* 2015, 28, 836-844.
8. WHO.Tumours of the uterine corpus. In: Kurman RJ, Carcangiu ML, Herrington CS, Young RH (eds) WHO classification of tumours of the female reproductive organs. 2014, 4th ed, pp 126–165.
9. TALHOUK, A. & MCALPINE, J. N.New classification of endometrial cancers: the development and potential applications of genomic-based classification in research and clinical care. *Gynecol Oncol Res Pract* 2016, 3, 14https:// DOI: 10.1186/s40661-016-0035-4.
10. MÜLLER, W., BURGART, L. J., KRAUSE-PAULUS, R., THIBODEAU, S. N., ALMEIDA, M., *et al.*The reliability of immunohistochemistry as a prescreening method for the diagnosis of hereditary nonpolyposis colorectal cancer (HNPCC)--results of an international collaborative study. *Fam Cancer* 2001, 1, 87-92https:// DOI: 10.1023/a:1013840907881.
11. BARROW, E., JAGGER, E., BRIERLEY, J., WALLACE, A., EVANS, G., *et al.*Semiquantitative assessment of immunohistochemistry for mismatch repair proteins in Lynch syndrome. *Histopathology* 2010, 56, 331-44https:// DOI: 10.1111/j.1365-2559.2010.03485.x.
12. DOGHRI, R., HOUCINE, Y., BOUJELBÈNE, N., DRISS, M.,CHARFI, L., *et al.*Mismatch Repair Deficiency in Endometrial Cancer: Immunohistochemistry Staining and Clinical Implications. *Appl Immunohistochem Mol Morphol* 2019, 27, 678-682https:// DOI: 10.1097/pai.0000000000000641.
13. LANDRUM, M. J., LEE, J. M., RILEY, G. R., JANG, W., RUBINSTEIN, W. S., *et al.*ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res* 2014, 42, D980-5https:// DOI: 10.1093/nar/gkt1113.
14. BALMAÑA, J., DIGIOVANNI, L., GADDAM, P., WALSH, M. F., JOSEPH, V., *et al.*Conflicting Interpretation of Genetic Variants and Cancer Risk by Commercial Laboratories as Assessed by the Prospective Registry of Multiplex Testing. *J Clin Oncol* 2016, 34, 4071-4078https:// DOI: 10.1200/jco.2016.68.4316.
15. TAVASSOLI, F. A. & DEVILEE, P. 2003. *Pathology and genetics of tumours of the breast and female genital organs*, Iarc.
16. GENESTIE, C., LEARY, A., DEVOUASSOUX, M. & AUGUSTE, A.[Histological and molecular classification of endometrial carcinoma and therapeutical implications]. *Bull Cancer* 2017, 104, 1001-1012https:// DOI: 10.1016/j.bulcan.2017.08.004.
17. DENSCHLAG, D., ULRICH, U. & EMONS, G.The diagnosis and treatment of endometrial cancer: progress and controversies. *Dtsch Arztebl Int* 2010, 108, 571-7https:// DOI: 10.3238/arztebl.2011.0571.
18. KANAME, T., YANAGI, K. & NARITOMI, K.A commentary on the promise of whole-exome sequencing in medical genetics. *Journal of human genetics* 2014, 59, 117-118.
19. VAN DIJK, E. L., AUGER, H., JASZCZYSZYN, Y. & THERMES, C.Ten years of next-generation sequencing technology. *Trends Genet* 2014, 30, 418-26https:// DOI: 10.1016/j.tig.2014.07.001.
20. SOSLOW, R. A., WEIGELT, B., CHANG, Y. S., HUANG, H. D., YEH, K. T., *et al.*Evaluation of whole exome sequencing by targeted gene sequencing and Sanger sequencing. *Histopathology* 2017, 471, 222-232https:// DOI: 10.1111/his.13240

21. BARETTI, M. & LE, D. T.DNA mismatch repair in cancer. *Pharmacol Ther* 2018, 189, 45-62https:// DOI: 10.1016/j.pharmthera.2018.04.004.

22. OLAVE, M. C. & GRAHAM, R. P.Mismatch repair deficiency: The what, how and why it is important. *Genes Chromosomes Cancer* 2022, 61, 314-321https:// DOI: 10.1002/gcc.23015.

23. DEDEURWAERDERE, F., CLAES, K. B., VAN DORPE, J., ROTTIERS, I., VAN DER MEULEN, J., *et al.*Comparison of microsatellite instability detection by immunohistochemistry and molecular techniques in colorectal and endometrial cancer. *Sci Rep* 2021, 11, 12880https:// DOI: 10.1038/s41598-021-91974-x.

24. REITSAM, N. G., MÄRKL, B., DINTNER, S., WAIDHAUSER, J., VLASENKO, D., *et al.*Concurrent loss of MLH1, PMS2 and MSH6 immunoexpression in digestive system cancers indicating a widespread dysregulation in DNA repair processes. *Front Oncol* 2022, 12, 1019798https:// DOI: 10.3389/fonc.2022.1019798.

25. RABBAN, J. T., CALKINS, S. M., KARNEZIS, A. N., GRENERT, J. P., BLANCO, A., *et al.*Association of tumor morphology with mismatch-repair protein status in older endometrial cancer patients: implications for universal versus selective screening strategies for Lynch syndrome. *Am J Surg Pathol* 2014, 38, 793-800https:// DOI: 10.1097/pas.0000000000000177.

26. KOH, W. J., ABU-RUSTUM, N. R., BEAN, S., BRADLEY, K., CAMPOS, S. M., *et al.*Uterine Neoplasms, Version 1.2018, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2018, 16, 170-199https:// DOI: 10.6004/jnccn.2018.0006.

27. DOULGERAKI, T., VAGIOS, S., KAVOURA, E., YIANNOU, P., MESSINI, I., *et al.*Mismatch repair status in high-grade endometrial carcinomas of endometrioid and non-endometrioid type. *J buon* 2019, 24, 2020-2027.

28. GONZÁLEZ VILLA, I., GONZÁLEZ DÁVILA, E. F., AFONSO, I. J. E., BLANCO, L. I. M., FERRER, J. F. L., *et al.*An effective algorithm to detect the possibility of being MSI phenotype in endometrial cancer given the BMI status and histological subtype: a statistical study. *Clin Transl Oncol* 2022, 24, 1809-1817https:// DOI: 10.1007/s12094-022-02837-4.

29. LIU, T., WANG, Y., WANG, Y. & CHAN, A. M.Multifaceted regulation of PTEN subcellular distributions and biological functions. *Cancers* 2019, 11, 1247

30. AGUILAR, M., CHEN, H., RIVERA-COLON, G., NIU, S., CARRICK, K., *et al.*Reliable Identification of Endometrial Precancers Through Combined Pax2, β-Catenin, and Pten Immunohistochemistry. *Am J Surg Pathol* 2022, 46, 404-414https:// DOI: 10.1097/pas.0000000000001810.

31. JARBOE, E. A. & MUTTER, G. L. Endometrial intraepithelial neoplasia. Seminars in diagnostic pathology, 2010. Elsevier, 215-225.

32. MUTTER, G. L., MONTE, N. M., NEUBERG, D., FERENCZY, A. & ENG, C.Emergence, involution, and progression to carcinoma of mutant clones in normal endometrial tissues. *Cancer research* 2014, 74, 2796- 2802.

33. MONTE, N. M., WEBSTER, K. A., NEUBERG, D., DRESSLER, G. R. & MUTTER, G. L.Joint Loss of PAX2 and PTEN Expression in Endometrial Precancers and CancerPAX2 and PTEN in Endometrial Neoplasia. *Cancer research* 2010, 70, 6225-6232https://.

34. TAKEICHI, M.Cadherins in cancer: implications for invasion and metastasis. *Curr Opin Cell Biol* 1993, 5, 806-11https:// DOI: 10.1016/0955-0674(93)90029-p.

35. SHIOZAKI, H., OKA, H., INOUE, M., TAMURA, S. & MONDEN, M.E-cadherin mediated adhesion system in cancer cells. *Cancer* 1996, 77, 1605-13https:// DOI: 10.1002/(sici)1097- 0142(19960415)77:8<1605::aid-cncr28>3.0.co;2-2.

36. CARICO, E., ATLANTE, M., GIARNIERI, E., RAFFA, S., BUCCI, B., *et al.*E-cadherin and alpha-catenin expression in normal, hyperplastic and neoplastic endometrium. *Anticancer Res* 2010, 30, 4993-7

37. WONG, A. S. & GUMBINER, B. M.Adhesion-independent mechanism for suppression of tumor cell invasion by E-cadherin. *J Cell Biol* 2003, 161, 1191-203https:// DOI: 10.1083/jcb.200212033.

38. GUMBINER, B. M.Cell adhesion: the molecular basis of tissue architecture and morphogenesis. *Cell* 1996, 84, 345-57https:// DOI: 10.1016/s0092-8674(00)81279-9.

39. PEĆINA-SLAUS, N.Tumor suppressor gene E-cadherin and its role in normal and malignant cells. *Cancer Cell Int* 2003, 3, 17https:// DOI: 10.1186/1475-2867-3-17.

40. JOEHLIN-PRICE, A. S., PERRINO, C. M., STEPHENS, J., BACKES, F. J., GOODFELLOW, P. J., *et al.*Mismatch repair protein expression in 1049 endometrial carcinomas, associations with body mass index, and other clinicopathologic variables. *Gynecol Oncol* 2014, 133, 43- 7https:// DOI: 10.1016/j.ygyno.2014.01.017.

41. 10.1016/j.cca.2017.06.015.