Histological study of endometrial hyperplasia for diabetic women in Erbil city

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Abstract

Objective: Endometrial hyperplasia and type 2 diabetes mellitus (T2DM) may be correlated, but this is questionable. This investigation's goal is to evaluate T2DM's role in the emergence of endometrial hyperplasia.

Methods: Samples were collected from diabetic women over the age of 40 who had undergone a dilatation and curettage (D&C) operation after having their endometrial hyperplasia identified by ultrasonography. All samples underwent histological evaluation and p53, IL-6, and ER immunohistochemistry staining (estrogen receptors)

Results: Endometrium hyperplasia samples that underwent histological analysis showed bleeding, infiltration of inflammatory cells, and complex endometrial gland hyperplasia as a result of epithelial cell proliferation. The histological examination of endometrial hyperplasia in diabetic patients revealed significant differences in simple hyperplasia between samples with and without atypia. When the two hyperplasia groups were compared, it was found that there were significant variations ($P< 0.05$) in the gland area, stromal area, gland/stromal ratio, gland density, gland diameter, and epithelial height.

Conclusion: Diabetes is associated with both atypic and non-atypic endometrial hyperplasia, according to histological and immunohistochemical investigations. In diabetic hyperplasia with atypia as opposed to diabetic hyperplasia without atypia, there is a higher expression of ER, IL-6, and P53.

INTRODUCTION

Endometrial hyperplasia (EH) is a uterine condition that encompasses a variety of endometrial morphological changes. When compared to normal proliferative endometrium, its main characteristic is a rise in the endometrial gland-to-stroma ratio [1]. Typically, persistent estrogen exposure causes EH. [2]. EH is often referred to an unchecked endometrial gland growth. It occurs when progesterone's inhibitory effects on endometrial cells are comparatively weak and estrogen's stimulation of that tissue is unopposed. [3]. Endometrial hyperplasia is a common disorder brought on by exposure to exogenous or endogenous estrogen as well as a relative lack of progesterone. [4].

In gynecology, endometrial hyperplasia is a frequent disorder that can also be a sign of endometrial cancer [1] which is the most frequent fatal gynecological cancer, develops in up to 40% of women with endometrial hyperplasia with atypia. [5] Diabetic may increase the risk of endometrial abnormality, according to mounting evidence [6]. The most prevalent kind of diabetes, Type 2 diabetic Mellitus (T2DM) which is brought on by high blood glucose levels. The main source of energy that is mostly derived from food is blood glucose. The pancreas produces the hormone insulin, which aids in the transport of

glucose into body cells for use as fuel. Increased insulin levels and a common dysregulation of ovarian steroid hormones are two processes that are shared by gynecologic malignancies, which are more common and have a significant incidence in T2DM-affected females [7].

T2DM and endometrial hyperplasia are both prevalent diseases, and they frequently co-occur in the same person [8]. The development of malignancies is thought to be strongly influenced by type 2 diabetic mellitus (T2DM) [9]. Therefore, it is crucial to understand the mechanisms behind these two diseases in order to pinpoint possible therapeutic molecular targets for the management of endometrial hyperplasia. Endometrial cancer and endometrial hyperplasia risk in T2DM-affected women is doubled and quadrupled, respectively [10]. The most obvious clinical symptom of T2DM is hyperglycemia, which promotes tumor growth through a variety of mechanisms that boost proliferative, anti-apoptotic, and metastatic cancer activity [11]. However, there may be biological connections between T2DM and the cancers.

All forms of hyperplasia share the traits of increased gland-to-stroma ratios, aberrant gland morphologies, and variations in gland size. Simple hyperplasia, which has little endometrial glandular crowding, complex hyperplasia, which has more endometrial glandular crowding, and atypical hyperplasia, which has complex glandular crowding, cytologic atypia, and a high risk of developing endometrial cancer, are some of the EH types that have been identified through classification based on architectural crowding and nuclear atypia.

In this work, diabetic women's endometrial hyperplasia's histology is the main subject.

Methods

The study was conducted between September 2021 and January 2022, samples were taken from women who firstly diagnosed endometrial hyperplasia by ultrasonography and undergone a dilatation and curettage (D&C) procedure, they were over the age of 40. Samples were obtained from the Erbil Governorate's Maternity Teaching Hospital.

70 samples were taken from women who had surgical dilatation and curettage (D&C). All samples underwent histological analysis and immunohistochemical staining with p53, IL-6, and ER (estrogen receptors)

The questionnaire was conducted, the purpose of the questionnaire was to gather data about the patient’s age, weight, blood type, menstrual cycle, menopause, bleeding, number of births, mother's age during her first pregnancy, type of delivery, number of miscarriages, number of dilation and curettage D&C and its iterating if present, diabetes, use of birth control pills, and chronic illness.

10% formalin was used to fix tissue samples for immunocytochemistry diagnosis or for histological analysis. Hematoxylin and eosin are a common stain used to show how the tissues are organized histologically [12]. In order to investigate the samples, 6 µ paraffin slices were made. To examine all of the samples, the manufacture procedure was followed when using the immunohistochemical stains, which included the tumor suppressor p53 (Dako), the ER pharmDxTM Kit, and IL6 (Thermo).

Tissue Morphometry

The Image-J® software was used to measure the tissue morphometry for different criteria that depended on the current study as follows

1- Immunohistochemistry expression

Light microscopy was used to examine the tissue slides at a 100x magnification power.

Twenty fields at random were selected on each slide, and each field was exposed to Image-J® software utilizing the following option. Choose object from Image>Layers>Counting. The total number of cells in each field was determined by counting all of the cells in that field. Afterward, using the same method as before, the cells that expressed a positive reaction to the antibody used in the IHC protocol were counted to determine the total number of cells in each field that displayed a positive reaction to a particular antibody (ER, IL-2, and P53).

(Table 1) and to express the values as scores and then these scoring data were subjected to statistical analysis.

Table 1: Microscopic scoring criteria for IHC and histopathological lesions

Criteria	0+	1+	2+	3+	4+
ER	Negative	Weak	+Positive	++Positive	+++Positive
IL-6	Negative	Weak	+Positive	++Positive	+++Positive
P53	Negative	Weak	+Positive	++Positive	+++Positive

2- Hemorrhages

To get the total area in one field, all tissue areas in each field were taken into account. Using the same method as before, the hemorrhaged area was then computed to determine the total area in each field that displayed hemorrhage. The mean bleeding area for each slide was later computed. The obtained information (area of hemorrhages) was then re-defined using the scoring table (Table 2), with the values expressed as scores, and these scoring data were then subjected to statistical analysis.

Table 2: Microscopic scoring criteria for hemorrhages

Criteria	0+	1+	2+	3+	4+
Hemorrhages	Absent	Few	Moderate	Intense	Massive

3- Inflammatory cells

To calculate the overall area of a field, all tissue areas in that field were taken into account. Next, the total number of inflammatory cells present in that field was calculated using the Image>Layers>Counting>Select object option. The total number of inflammatory cells in the calculated field divided by the total number of calculated fields, with the result reported as cells/m2, was used to compute the mean of inflammatory cells for each slide. Then, using the SPSS version 22.0 program, statistical analysis was performed on the data.

4- Hyperplasia calcification and scoring

A- Endometrium Hyperplasia Calcification: Types of hyperplasia can be categorized into the following groups according to the WHO 2014 classification, which was approved by the International Society of Gynecological Pathologists:

-Simple hyperplasia: although there were more glands, the normal architecture was unaffected. When hypochromatic nuclei in the abnormal glands were somewhat enlarged and stratification in the hyperplastic glands was lost, they appeared alongside normal endometrial glands.

-Complex hyperplasia: When endometrial glands display a high degree of intricacy and are arranged irregularly and close to one another with little or no stroma and tufted intraluminal projections, this is a sign.

-Proliferative hyperplasia: This type of hyperplasia is characterized by an increase in

the number of glands with a well-defined circular shape and normal tissue architecture.

B- Endometrium Hyperplasia Scoring

A random sample of 20 fields were selected for each slide, and the number of glands present in each field was tallied. Following this, the mean number of hyperplastic glands present in each field was computed. Following a score table (table 3), the data gathered in the preceding stage were subjected to statistical analysis.

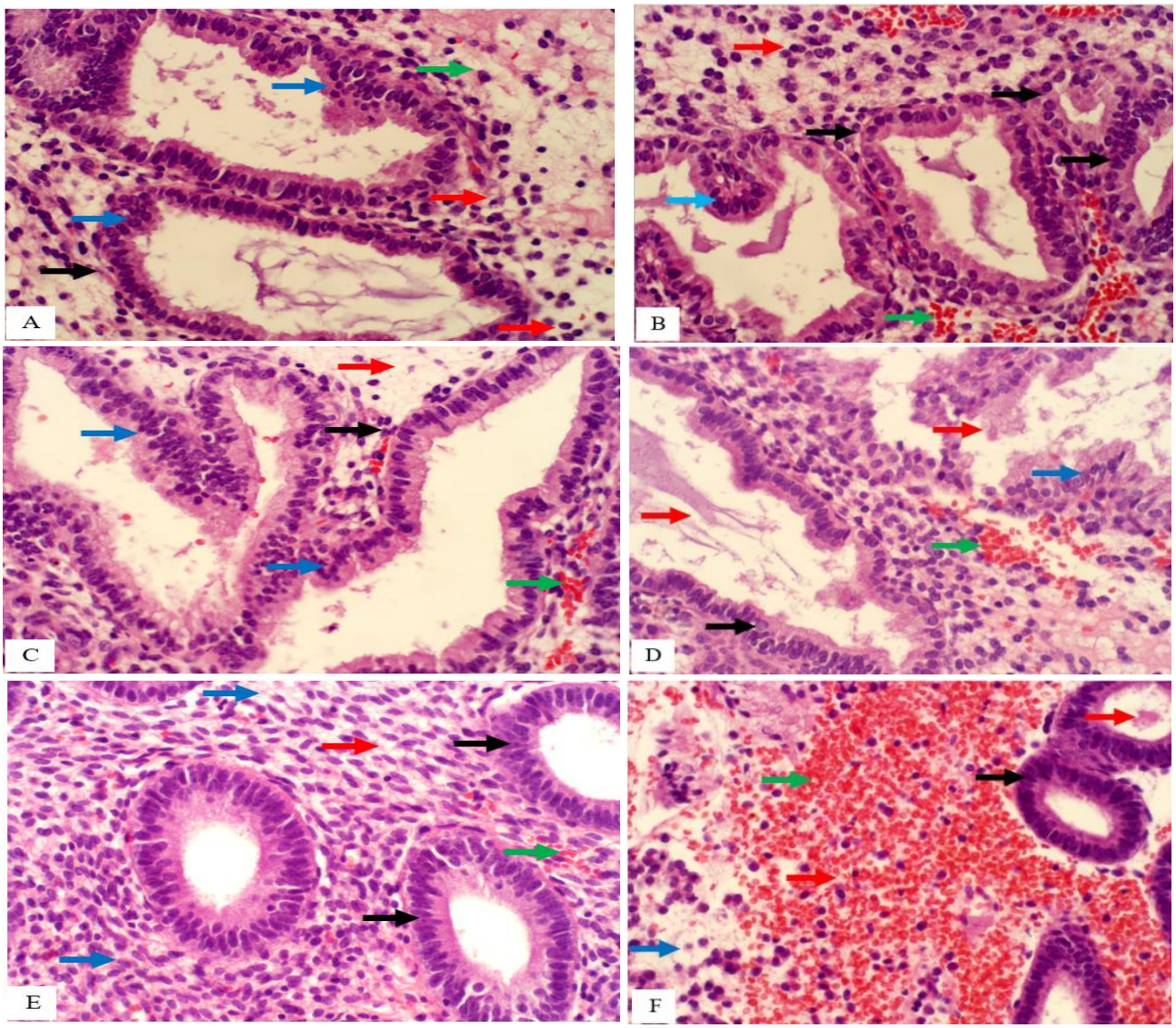
Table 3: Microscopic scoring criteria for hemorrhages

Criteria	0+	1+	2+	3+	4+
Simple	Absent	1-3	4-7	8-10	More 10
Complex	Absent	1-3	4-7	8-10	More 10
Proliferative	Absent	1-3	4-7	8-10	More 10

Results:

Only 30 of the 70 samples that were gathered for this study contained diabetics. Histological examinations revealed changes in the tissue's texture, including epithelial cell proliferation that results in complicated hyperplasia of endometrial glands, infiltration of inflammatory cells and hemorrhage in endometrium hyperplasia samples Figure (1)

Figure 1: without atypia endometrium hyperplasia A- showed complex hyperplasia of endometrial glands (black arrow), B- hyperplasia of epithelial cells lining the endometrial glands (blue arrow), C- hyperplasia of epithelial cells lining these glands which appear as figures inside the lumen (blue arrows), few stromal extracellular frameworks (red arrow) , D- deposition of eosinophilic material in gland lumen (red arrow) and hemorrhages (green arrow). E- abundant stromal extracellular matrix (blue arrow), vacuolar degeneration (red arrow), with hemorrhages (green arrow) and F- proliferative hyperplasia of endometrial glands (black arrow), vacuolar degeneration (blue arrow), deposition of eosinophilic material (red arrow), with hemorrhages (green arrow). H&E. 400x.

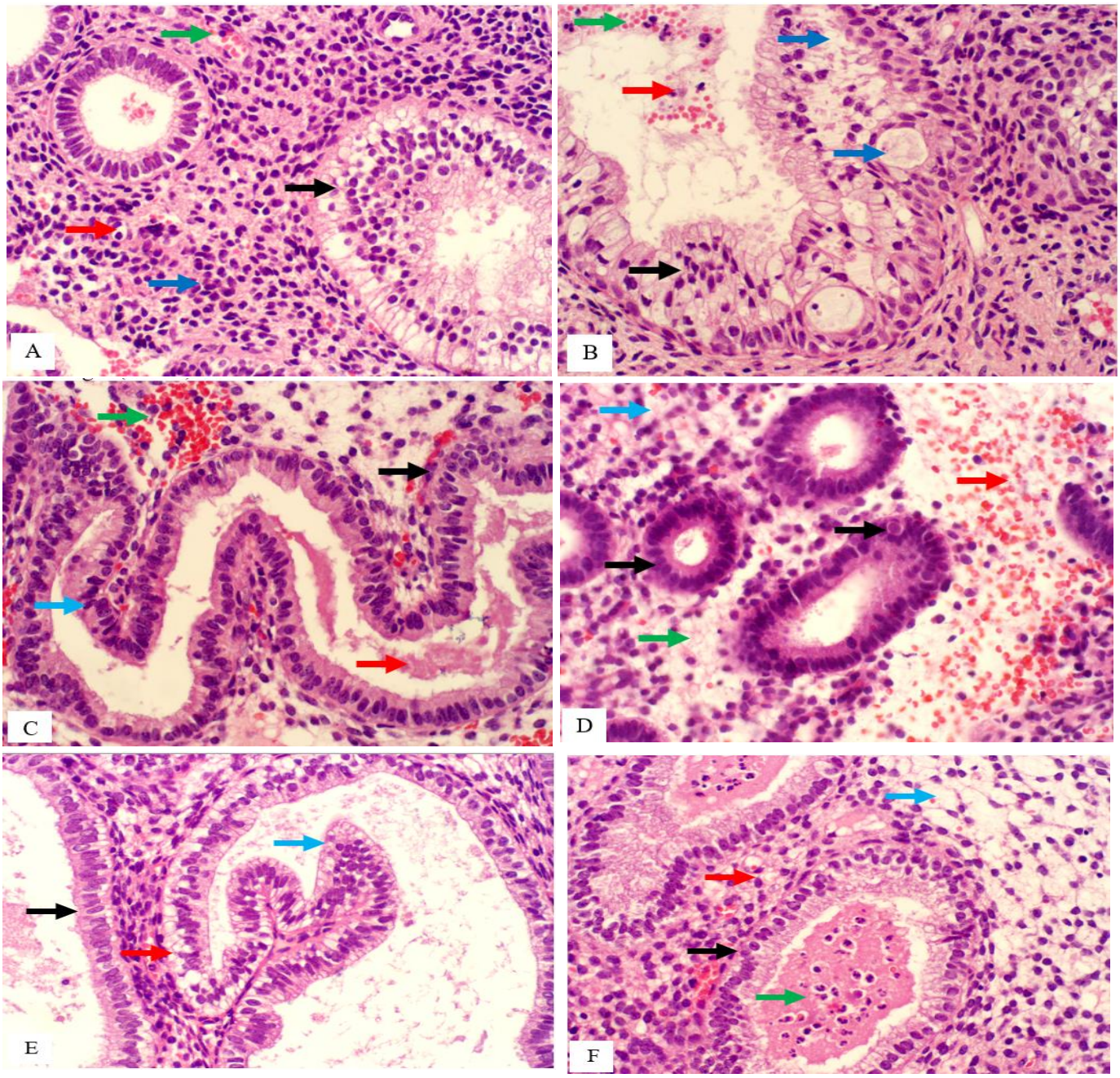


Some of the diabetic samples displayed endometrium hyperplasia with atypia; in addition, these samples displayed a significant alteration in histological texture, including proliferative hyperplasia of endometrial glands epithelia, few stromal extracellular matrix, vacuolar degeneration, infiltration of inflammatory cells, deposition of eosinophilic

material, desquamination of necrotic of necrotic epithelial cells in the gland lumen.

Figure (2)

Figure 2: showed atypical endometrium hyperplasia A- complex hyperplasia of endometrial glands with atypia (black arrow) infiltration of inflammatory cells (blue arrow), vacuolar degeneration (red arrow), congestion of blood vessels (green arrow), B- vacuolar degeneration (blue arrow), deposition of eosinophilic material (red arrow), with congestion (green arrow).C- deposition of eosinophilic material in gland lumen (red arrow) and hemorrhages (green arrow), D- few stromal extracellular matrix (blue arrow), vacuolar degeneration (red arrow), with hemorrhages (green arrow), E), the epithelial cells appear as a projection in the lumen of cystically dilated glands (blue arrow), vacuolar degeneration (red arrow) and F- infiltration of multinucleated leukocytes in the lumen (green arrow). H&E. 400x



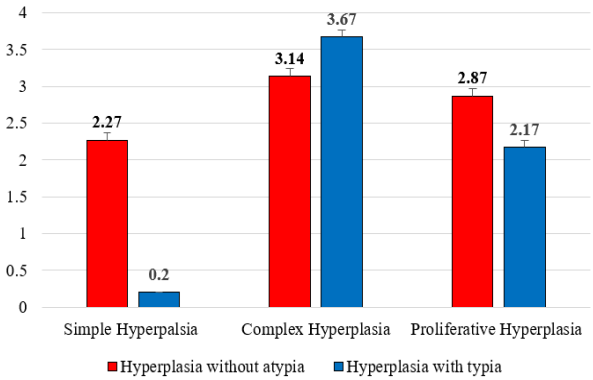
The statistical analytical data of histological examination of endometrial hyperplasia in diabetic patient showed significant differences in simple hyperplasia between hyperplasia with atypia and without atypia samples while the other data of the study including amount of inflammatory cell, Hemorrhage, complexity of hyperplasia and its proliferation showed no significant differences. (Table 1Figure 3)

Table 1: The different horizontal letters mean a significant differences at P<0.05.

Inflammatory cell (cell/m^2)	138.14±12.04 **A**	139.72±10.27 **A**
Hemorrhages	2.77±0.11 **A**	2.81±0.21 **A**
Simple hyperplasia	2.27±0.18 **A**	0.20±0.01 **B**
Complex hyperplasia	3.14±0.21 **A**	3.67±0.17 **A**
Proliferative hyperplasia	2.87±0.85 **A**	2.17±0.42 **A**

The statistical analysis using SPSS version 22.0, a One-way analysis of variance test used to calculate the significant differences between tested groups at P<0.05

Figure 3: showed significant differences in simple hyperplasia between hyperplasia with atypia and without atypia



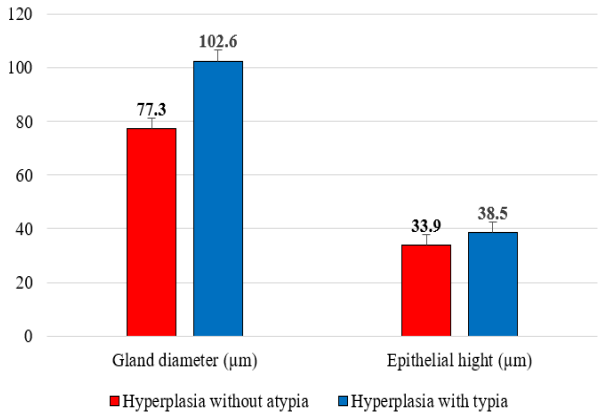
The results of comparison the two hyperplasia groups (with and without atypia) showed significant differences in gland area, stromal area, gland/ stromal ratio, gland density, gland diameter and epithelial Hight. (Table 2,Figure 4)

Table 2: The different horizontal letters mean a significant differences at P<0.05.

gland area (µm^2)	98122.88±6606.1 **B**	110746.27±5671.7 **A**
stroma area (µm^2)	913915.47 ±6416.5 **B**	1069428.54 ±3334.3 **A**
gland/stroma ratio (%)	1/9.3 **B**	1/10.7 **A**
gland density (4xfield)	15.32±0.6 **B**	17.54±0.9 **A**
gland diameter (µm)	77.3±5.9 **B**	102.6±4.7 **A**
Epithelial Hight (µm)	33.9±2.2 **B**	38.5±4.0 **A**

The statistical analysis using SPSS version 22.0, a One-way analysis of variance test used to calculate the significant differences between tested groups at P<0.05

Figure 4: showed significant differences in gland area and epithelial Hight



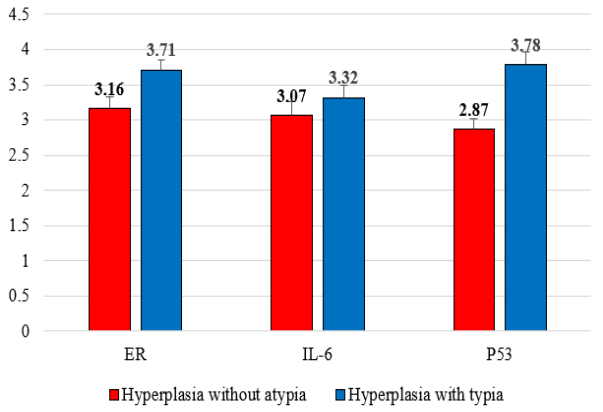
The immunobiological studies of the samples showed significant differences in expression of estrogen receptor ER, IL6 and P53 antibodies. As its clearly seen in table 3 and figure 5

Table 3: The different horizontal letters mean a significant differences at P<0.05.

Group	Hyperplasia with diabetic	
	Without atypia	**With atypia**
ER expressions	3.16±0.17 **B**	3.71±0.14 **A**
IL-6 expressions	3.07±0.19 **B**	3.32±0.17 **A**
p53 expressions	2.87±0.14 **B**	3.78±0.19 **A**

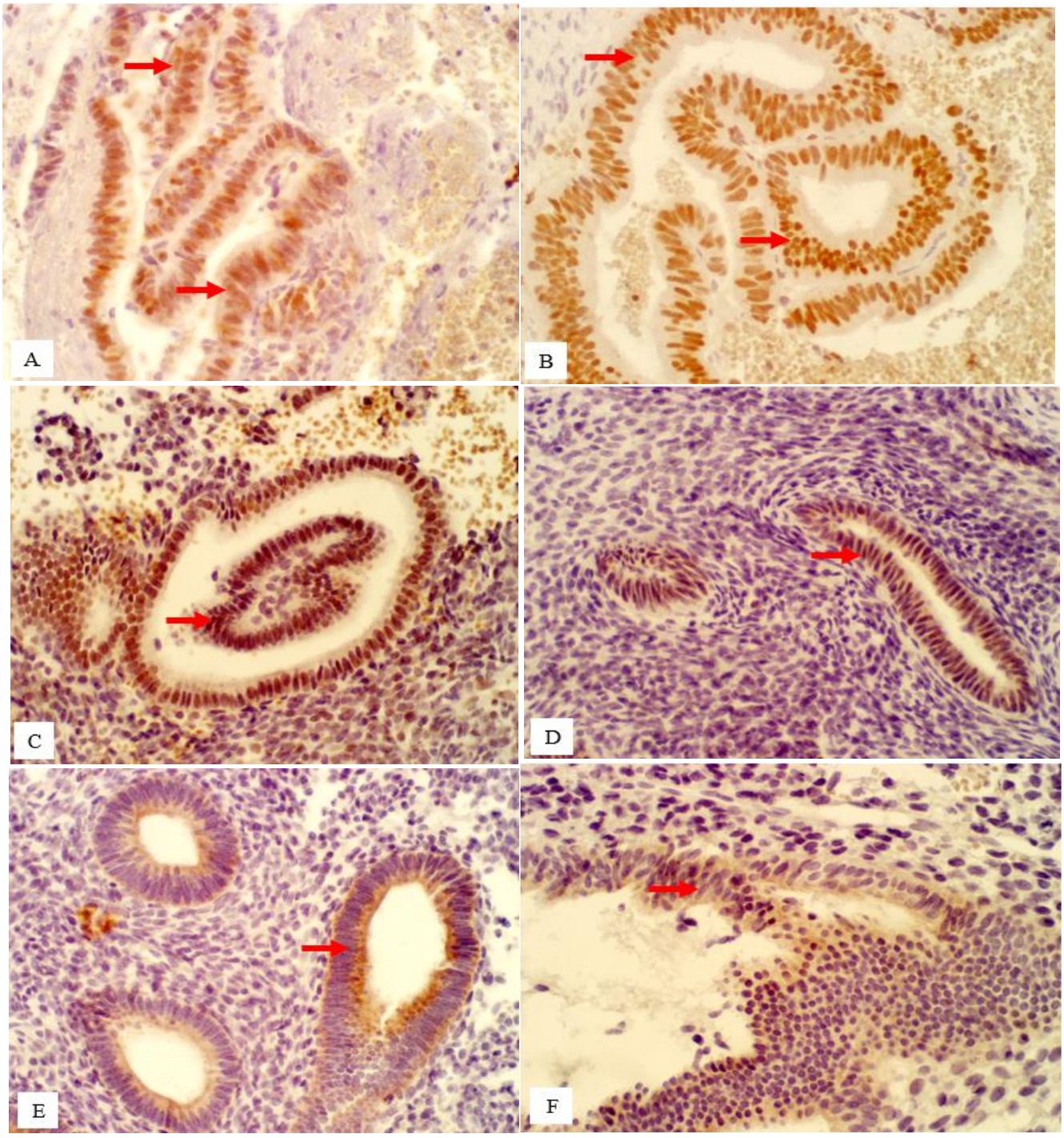
The statistical analysis using SPSS version 22.0, a One-way analysis of variance test used to calculate the significant differences between tested groups at P<0.05

Figure 5: showed significant differences in expression of estrogen receptor ER, IL6 and P53 antibodies.



These distinctions are evident in the immunobiological slide sections, where endometrial sections react positively to the ER, P53, and IL6 antibodies. (Figure 6)

Figure 6: Immunohistochemical reaction in the diabetic endometrium hyperplasia samples. A &B Showed positive staining with ER antibodies in the nucleus as golden-brown granules (arrow). C &D Showed positive staining with P53 antibodies in the nucleus as golden-brown dots (arrow) and E&F Showed positive staining with IL-6 antibodies in the cytoplasm as golden-brown granules (arrow) IHC. 400x



Discussion:

In gynecology, endometrial hyperplasia is a frequent disorder that can also be a sign of endometrial cancer [1]. There is mounting evidence that T2DM raises the risk of endometrial cancer. incidence of cancer [6]. T2DM and endometrial hyperplasia are both prevalent diseases, and they frequently co-occur in the same person [8]. The hallmarks of endometrial hyperplasia include abnormal cell proliferation and expansion [13]. This study highlighted the T2DM role in alteration the texture of endometrium as it clears in the H&E sections (figure 1&2). Where endometrial glands showed complex hyperplasia, deposition of eosinophilic material in gland lumen, hemorrhage, and vacuolar degeneration. The considerable increase in endometrial proliferation raised the possibility that T2DM might affect the outcome of endometrial hyperplasia. The findings of this study are in agreement with experimental animal investigation results [14]. According to a molecular biological screen and validation investigation, A member of the glycosyltransferase 2 protein family which is encoded by GALNT2 gene may be the essential protein connecting T2DM and endometrial hyperplasia. GALNT2 participates in the O-glycosylation of proteins involved in lipid metabolism, which has been proven in human genetic research to be a regulator of mammalian lipid metabolism that mostly affects high-density lipoprotein [15]. According to research, T2DM is characterized by abnormal glycosylation, which has an impact on a variety of cellular functions, including cell division, apoptosis, differentiation, transformation, migration, invasion, and immunological responses [16].

The study statistical analytical data of histological findings of endometrial hyperplasia in diabetic patient showed significant differences in simple hyperplasia between hyperplasia with atypia and without atypia samples while the other data of the study including amount of inflammatory cell, Hemorrhage, complexity of hyperplasia and its proliferation showed no significant differences. (Table 1Figure 3)

The results of Immunohistochemical examination showed different expressions of antibodies between the diabetic sections of atypia and without atypia endometrial hyperplasia, Table 3.

The Estrogen receptors (ER) found in epithelial, stromal, and vascular cells are the mechanism through which Estrogenic effects work. In the stroma of the functional and basal layers of the endometrium, as well as in the epithelial glands, ER protein is expressed. In the stromal and epithelial cells of the endometrium during the secretory phase, progesterone causes the expression of ER to be downregulated [17]. Gynecological diseases may be influenced by the endometrium's expression of steroid receptors. Endometrial epithelial cells' mid-secretory phase downregulation of ER has been linked to uterine receptivity deficiencies like luteal phase defect, endometriosis, and hyperplasia [18]. According to a study, ER was mostly expressed in glandular epithelial cells, however it was also found in stromal cells, during the menstrual cycle [19]. that was agree with the results of the study (figure 6 A&B).

P 53 is known as "the defender of the genome" because it blocks the growth of cells with damaged DNA and has emerged as a key protein in cancer. According to research, endometrial glands close to endometrial cancer express p53, which is linked to endometrial hyperplasia [20]. It is difficult to evaluate p53 over-expression using immunochemistry because the antibody being used cannot distinguish between the wild-type and mutant forms of the protein. This makes the staining pattern in these situations extremely important because only intense, diffuse staining can reliably predict the

presence of mutation in the p53 gene. endometrial hyperplasia instances. It was believed that p53 expression resulted from wild type p53 being elevated to repair DNA damage rather than from a buildup of the stable mutant form [21]. As the severity of the endometrial lesion advanced from EH to endometrial carcinoma, p53 expression increased. That was accordant with study which was spotlight on significant differences between hyperplasia with atypia and without atypia endometrium sections as its clearly seen in the table 3 and Figure 5.

The endometrial epithelia's brown precipitate contained an expression of the IL-6 protein. positive staining was seen in the cytoplasm of surface epithelial cells, lamina propria cells, and in endometrial gland cells from mucosa layer. The study finding concerning to the IL6 expression showed significant differences between endometrial hyperplasia with atypia and without atypia as it illustrated in figure 5and table 3.

The interleukin-6 is a cytokine that has been shown to regulate hematopoiesis, induce acute phase reactions and inflammation [22]. It also plays a role in T cells immunological responses. Studies bespoken that the human immune response is differentiated and regulated by the interleukin -6, as are a number of cellular processes such as angiogenesis, apoptosis, and proliferation [23] [24]. Endothelial cells are a type of cells that reported to produce IL-6. According to the immunohistochemical analysis of the current study, diabetic patients with atypia endometrial hyperplasia had higher levels of IL-6 expression than sections without atypia. Years before the disease manifests, those who develop type 2 diabetes show signs of low-grade inflammation. Low-grade irritation like this is thought to play a role in the pathogenetic mechanisms that lead to type 2 diabetes.The findings of this investigation corroborated with the study of [25].

Conclusion:

Both atypic and non-atypic endometrial hyperplasia are affected by diabetes, according to histological and immunohistochemical studies. The expression of ER, IL-6, and P53 are stronger in diabetic hyperplasia with atypia than diabetic hyperplasia without atypia.

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