***Significance of Sex Determining Region Y-Box transcription factor 4 (SOX4) expression and Nuclear morphometry in endometrial carcinoma***

**Abstract**:

**Background**: Endometrial carcinoma (EC) is the 6th most common cancer worldwide. SOX4 has been used as an immunohistochemical marker. Expression of SOX4 by cancer cells has been demonstrated in EC. Nuclear morphometry is an imaging technique that plays important roles in endometrial hyperplasia (EH) and EC. **Aim**: This work aims to evaluate SOX4 expression and nuclear morphometry in EH and EC and the relation between its expression and different clinicopathological variables. **Material and method:** This is a selected retrospective study including 60 different cases of EC and EH. SOX4 immunostaining and nuclear morphometry were performed for all cases. **Results**: There was a highly significant statistical difference between SOX4 expression in studied cases according to type, FIGO grade, depth of myometrial invasion, LVSI (lymphovascular space invasion), T/B (Tumor/Border) configuration, desmoplasia, mitotic count, T, N, M components, TNM and FIGO stage (P<0.001, <0.001, <0.001, <0.001, <0.001 <0.001, <0.001, <0.001, =0.003, <0.001, <0.001 and <0.001 respectively) and highly significant statistical relation between MNA and clinicopathological parameters of studied cases. **Conclusion**: strong SOX4 expression was associated more with SEC (serous endometrial carcinoma), higher grade of EC, deeper myometrial invasion, LVSI, infiltrative tumor border, evidence of desmoplasia, higher mitotic count T, N, M components, TNM and FIGO stage. Nuclear morphometry is useful in diagnosis and differentiation of EC from premalignant lesions. SOX4 and Nuclear morphometry can be used together to diagnose and predict the outcome of EC.

**Keywords**: Endometrial hyperplasia; endometrial carcinoma; SOX4; nuclear morphometry.

**Introduction**:

Endometrial carcinoma is one of the deadliest cancers in the world with an increasing incidence and disease-associated mortality. It is the 2nd most common gynaecological cancer and the 6th most common malignant tumour in females worldwide **(1)**. According to Egyptian National Cancer institute, it is third most common gynaecological cancer. It represents 22.83% of female genital system malignancy and 72.37% of primary uterine corpus malignancy in Egypt **(2).**

Endometrial carcinoma is a multifactorial disease. The risk factors are classified into non-modifiable and modifiable risk factors including age, race, early menarche, late menopause, hereditary syndromes unopposed excess oestrogen, Type II diabetes mellitus, oestrogen-producing ovarian tumours, lifestyle and Alcohol **(3).**

According to the 5th edition WHO classification, endometrial hyperplasia (EH) is considered a precursor lesion of EC and classified according into hyperplasia without atypia and atypical endometrial hyperplasia (AEH)/ endometrioid intraepithelial neoplasia (EIN). EC is classified into several subtypes including endometrioid, serous, clear cell type, undifferentiated/dedifferentiated, mixed cell type and carcinosarcoma. Some subtypes have been added to the classification such as mesonephric-like adenocarcinoma and gastric (gastrointestinal-type) mucinous carcinoma **(4).**

Endometrioid carcinoma is classified into four groups according to TCGA molecular classification. They include ultra-mutated endometrial cancer with mutations in the exonuclease domain of DNA polymerase epsilon (POLE-mutated), tumours with microsatellite instability (MSI) (hypermutated), tumours harbour TP53 mutations with high copy number alterations and tumours with no specific molecular profile (NSMP) **(5)**

Sex Determining Region Y-Box transcription factor 4 (SOX4) belongs to C subgroup of SOX family. It has a role in development, cell differentiation and tumorigenesis. It is overexpressed in several human cancers, including breast, prostate, bladder and lung cancers **(6),** but its role in EC is not elucidated yet.

Nuclear morphometry is a quantitative objective and reproducible results that could be a useful supplement in diagnosis, prediction of prognosis and treatment planning for certain types of cancer using nuclear parameters including the mean and standard deviations (SD) of the nuclear area (MNA), longest (MLNA) and shortest (MSNA) nuclear Axis **(7). However,** its role is not fully elucidated in endometrial lesions.

**Aim of the work**

This study aims at conducting Immunohistochemical evaluation of (SOX4) expression in EC and precursor lesions and its correlation with histopathological features. Also, assessing the role of nuclear morphometry and investigating the correlation of immunohistochemical and morphometric findings trying to assess their combined possible role in endometrial carcinoma.

**Material and methods:**

**Study group:**

This is a retrospective study including 60 cases of endometrial lesions as follow: 37 cases of endometrioid endometrial carcinoma (EEC), 7 cases of serous endometrial carcinoma (SEC) and sixteen cases of endometrial hyperplasia of which 9 cases of AEH and 7 cases EH without atypia.

The material included archival formalin fixed paraffin embedded blocks processed during the years 2010 to 2015 as well as stained Hematoxylin and Eosin (H&E) slides for review. The blocks were collected from Department of Pathology and Early Cancer Detection Unit; Faculty of Medicine, Benha University, Egypt. Clinicopathological data were collected from the files of patients. Being a retrospective study, a written informed consent was not needed.

The cases were selected according to the availability of paraffin blocks and clinical records.  The study was approved by the Research Ethical committee of Faculty of Medicine, Benha University, Egypt (MSC 26/1/2022).

**Histopathological examination:**

Re-evaluation of sections from all selected cases was performed. The cases were re-evaluated for their type according to fifth edition of the WHO Classification of tumors of the uterine corpus **(4)**, graded according to FIGO grading and staged according to FIGO and TNM staging systems **(8 and 9).**

Evaluation of mitosis count was carried out using Leica ICC50 HD light microscope with wide angle (field diameter: 0.636 mm2; Leica ICC50 HD, Germany). The mitotic count was calculated as total number of figures counted in per 2 mm2 **(10).** T/B (Tumor/Border) configuration was categorized as infiltrative or pushing tumor border **(11and 12)** and presence of surrounding desmoplastic stromal reaction was also recorded **(13).**

**Immunohistochemical studies:**

Slides were stained according to manufacturer’s instructions with SOX4 rabbit polyclonal antibody (**ABclonal, USA**) at a dilution 1:50 at 4°C overnight. Immunodetection was carried out using a standard labelled streptavidin-biotin system (**Genemed, CA 94080, USA, South San Francisco**). Antigen retrieval was done by using solution of 10 mmol/L of Tris/EDTA (pH 9.0) and heating for 3 cycles, 10 minutes each in the microwave. Freshly prepared chromogen diaminobenzene (DAB, Envision TM Flex /HRP-Dako, REF K 8000) was used. Negative (Phosphate- buffered Saline) and positive control (breast and colonic cancer tissue) were enclosed in each run **(14and 15).**

**Interpretation of SOX4 expression:**

Positivity was considered as brownish homogenous nuclear staining of tumor cells **(16)**~~.~~ The expression level of SOX4 was defined by the sum of the staining-intensity and staining-extent scores as follows: “0” (negative, score of 0), “1” (weakly positive, score of 1-2), “2” (positive, score of 3-4), and “3” (strongly positive, score of 5-6) **(17).**

**Nuclear morphometry is performed as follow:**

Morphometric analysis was carried out by using Olympus® software imaging system (analySIS® LifeScience Series, Olympus Corporation, Hamburg, Germany) **(18)**. The morphometric analysis was carried out on H&E-stained slides to measure the nuclear parameters blindly regarding the histopathologic diagnosis **(7)**. Approximately 50 nuclei were examined in the most representative areas per slide with non-overlapping nuclei and their contours were traced manually using 40 x objectives to measure mean nuclear area **(19).**

**Statistical analysis**

Data analysis was performed by SPSS software, version *25* (SPSS Inc., PASW statistics for windows version *25*. Chicago: SPSS Inc.). Data was presented and suitable analysis was done according to the type of data obtained for each parameter. Mean, standard deviation (± SD), median, standard error (±SE), and range were used for description of numerical data. Frequency and percentage were used for description of non-numerical data. The significance of the obtained results (p value) was considered at the (≤0.05) level.

**Results**:

**Clinicopathological results:**

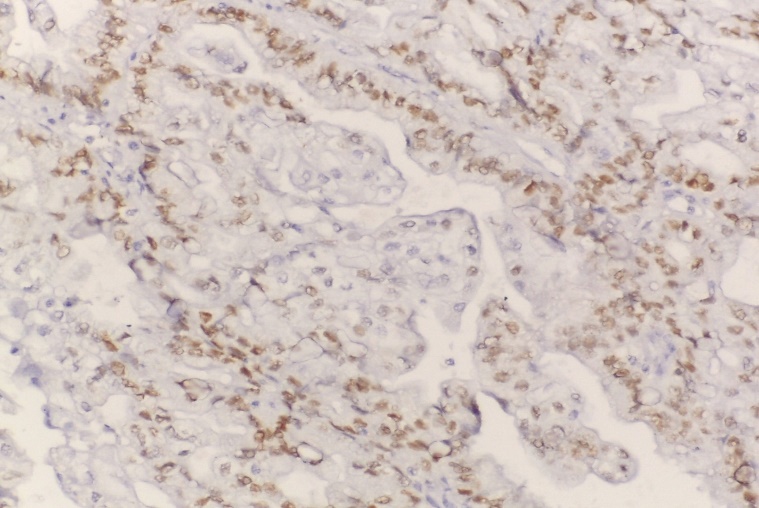
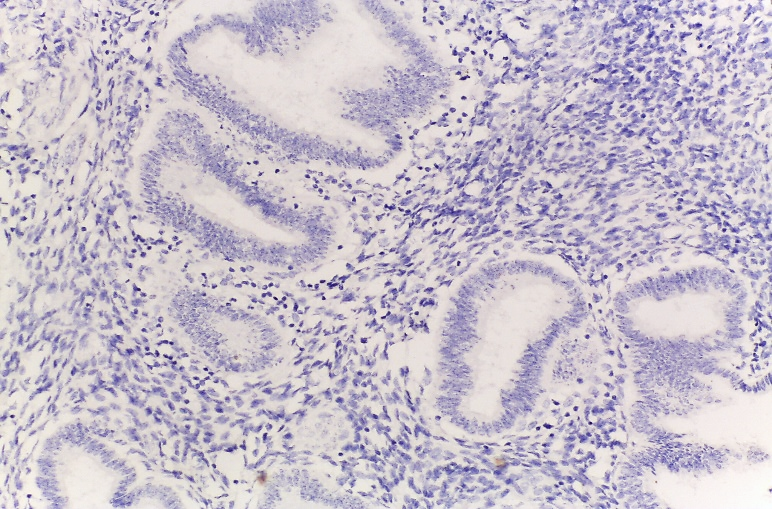
The age of 60 studied cases ranged from 42-75 years with the mean age was 57.33±10.56. endometrial carcinoma cases included 54.5 % of the studied patients < 55 years old while 45.5% were ≥55 years old (Mean±SD 55±7.21). The characteristics of the patients and tumors are listed in **(Table 1)**

**Immunohistochemical Results:**

**SOX4 expression in studied groups:**

For EH cases, all cases were SOX4 negative, 32 % of EC cases were of score 0, 11% were of score 1, 34% were of score 2 and 23% were of score 3. There was a statistically significant difference of SOX4 expression in studied groups (P<0.001).

**Relation between SOX4 expression score and different clinicopathological parameters of studied EC cases:**

****There was a statistically significant relation between SOX4 expression score and; histopathological type, FIGO grade, depth of myometrial invasion, LVSI, T/B configuration, Evidence of desmoplasia, mitotic count, T, N, M components, TNM and FIGO stage **(Table 1&Figure 1)**

|  |
| --- |
| A  B    D  C    E |

**Figure (1): Immunohistochemical expression of SOX4 in studied cases: (A): EH showing negative expression of SOX4, score 0. (B) EEC, FIGO grade I, positive for SOX4 with score 1. (C): EEC, FIGO grade II positive for SOX4 with score 2. (D): EEC, FIGO grade III, positive for SOX4 with score 3. (E): SEC, positive for SOX4 with score 3. (Streptavidin-biotin / DAB, x200)**

**Nuclear Morphometry results:**

**Relation between MNA results in EH studied and EC cases:**

The MNA among AEH cases was higher than EH without atypia cases (73.17±1.68 versus 60.37±2.29 µm2) with statistically significant difference between them (P<0.001). The MNA among EH cases was lower than EC cases (67.57±6.83 versus 134.93±46.31 µm2) with a significant difference of MNA between EH and EC cases (P<0.001).

**Relation between morphometric results and the clinicopathological parameters of EC:**

There was a statistically significant relation between MNA and Histopathological type, FIGO grade, depth of myometrial invasion, LVSI, T/B configuration, desmoplasia, mitotic count, T, N, M components, TNM and FIGO stage **(Table 1& Figure 2).**

|  |
| --- |
| A microscope view of a cell  Description automatically generatedA close-up of a microscope  Description automatically generated  B  A  A microscope view of a cell  Description automatically generatedA microscope view of a human tissue  Description automatically generated  D  C  A microscope view of a human tissue  Description automatically generated  E  F  A microscope view of a human tissue  Description automatically generated |

**Figure (2): MNA in studied cases:** **(A): Nuclear area of EH without atypia with mean value 58.73 µm2** **(B): Nuclear area of AEH with mean value 72.58µm2**  **(C): Nuclear area of EEC, FIGO grade I with mean value 103.05µm2**  **(D): Nuclear area of EEC, FIGO grade II with mean value 123.86 µm2**  **(E): Nuclear area of EEC, FIGO grade III with mean value 140.27 µm2**  **(F):** **Nuclear area of SEC, with mean value 236.66µm2 (H&E x400 with 100 zooming).**

**Table (1): Relation between SOX4 expression score, MNA and different clinicopathological parameters of studied EC cases:**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Clinicopathological parameters | SOX4 score | | | | | p value | MNA (µm2) | p value |
| **Total**  **n=44(%)** | **0**  **n=14(%)** | **1**  **n=5(%)** | **2**  **n=15(%)** | **3**  **n=10(%)** |
| Age/years  <55  ≥55 | 24(55)  20(45) | 10(42)  4(20) | 2(8)  3(15) | 9(37.5)  6(30) | 3(12.5)  7(35) | P=0.200 | 121.64±32.72  147.52±57.03 | P=0.07 |
| Histopathological type  EEC  SEC | 37(84)  7(16) | 14(38)  0 | 5(13.5)  0 | 15(40.5)  0 | 3(8)  7(100) | P<0.001\* | 116.47±13.89  232.51±31.64 | P<0.001\* |
| FIGO grade  I  II  III | 14(32)  20(45)  10(23) | 9(64)  5(25)  0 | 5(36)  0  0 | 0  15(75)  0 | 0  0  10(100) | P<0.001\* | 100.86±5.07  120.74±5.08  204.29±52.21 | P<0.001\* |
| Depth of myometrial invasion  <50%  ≥50% | 23(52)  21(48) | 13(56)  1(4) | 5(22)  0 | 5(22)  10(48) | 0  10(48) | P<0.001\* | 107.24±9.57  162.075±4.18 | P<0.001\* |
| LVSI  Present  Absent | 13(30)  31(70) | 0  14(45) | 0  5(16) | 4(31)  11(36) | 9(69)  1(3) | P<0.001\* | 184.07±59.19  112.16±11.99 | P<0.001\* |
| T/B configuration  Infiltrative  Pushing | 24(55)  20(45) | 3(12.5)  11(55) | 0  5(25) | 11(46)  4(20) | 10(41.5)  0 | P<0.001\* | 157.49±53.82  107.02±10.09 | P<0.001\* |
| Desmoplasia  Present  Absent | 30(68)  14(32) | 4(13)  10(71.5) | 2(6.5)  3(21.5) | 14(47)  1(7) | 10 (33.5)  0 | P<0.001\* | 146.98±51.02  104.31±8.12 | P=0.004\* |
| Mitotic count  ≥10/2 mm2  <10/2 mm2 | 15(34)  29(66) | 0  14(48) | 0  5(17) | 5(33)  10(35) | 10(67)  0 | P<0.001\* | 177.19±57.74  110.76±11.06 | P<0.001\* |
| T component  T1  T2  T3  T4 | 9(20)  13(30)  16(36)  6(14) | 8(89)  5(38)  1(6)  0 | 1(11)  4(31)  0  0 | 0  4(31)  11(69)  0 | 0  0  4(25)  6(100) | P<0.001\* | 98.71±3.85  109.64±9.79  123.40±5.65  198.27±53.53 | P<0.001\* |
| N component  N0  N1  N2 | 33(75)  9(20)  2(5) | 14(42.5)  0  0 | 5(15)  0  0 | 11(33.5)  4(44.5)  0 | 3(9)  5(55.5)  2(100) | P=0.003\* | 122.47±38.82  153.49±48.79  223.53±51.26 | P=0.002\* |
| M component  M0  M1 | 37(84)  7(16) | 14(38)  0 | 5(13.5)  0 | 15(40.5)  0 | 3(8)  7(100) | P<0.001\* | 114.66±12.87  232.51±31.63 | P<0.001\* |
| TNM stage group  Group 1  Group 2  Group 3  Group 4 | 9(21)  12(27)  15(34)  8(18) | 8(89)  5(42)  1(7)  0 | 1(11)  4(33)  0  0 | 0  3(25)  12(80)  0 | 0  0  2(13)  8(100) | P<0.001\* | 98.71±3.85  111.07±7.29  125.70±6.27  220.38±45.10 | P<0.001\* |
| FIGO stage  I  II  III  IV | 9(21)  12(27)  15(34)  8(18) | 8(89)  5(42)  1(7)  0 | 1(11)  4(33)  0  0 | 0  3(25)  12(80)  0 | 0  0  2(13)  8(100) | P<0.001\* | 98.71±3.85  111.07±7.29  125.70±6.27  220.38±45.10 | P<0.001\* |

**MC: Monte Carlo test, t: Student, F: One Way ANOVA test, \*statistically significant**

**Correlation between SOX4 expression score and nuclear morphometric results in studied EC cases:**

A statistically significant positive correlation was detected between SOX4 and MNA (r=0.852) with P value <0.001.

**Discussion:**

The current study revealed a statistically significant difference in SOX4 expression being only expressed in tumor cells suggesting that SOX4 may have a role in development of EC. SOX4 acts as an oncogene in activation of several pathways as PI3K/Akt and WNT/β-catenin pathways, which play a significant role in development of EC **(20).**

This agreed with studies on EC, lung adenocarcinoma and esophageal squamous cell carcinoma, revealing that SOX4 was overexpressed in these tumors compared to normal tissues, respectively (**21,22,23 and24)**.

This result contradicted to study of EC reporting that SOX4 is overexpressed in EH cases suggesting that high expression of SOX4 may lead to cellular transformation of endometrial lining (**25)**. This could be explained by using different technique to evaluate the expression levels of SOX4 gene by RT-PCR.

In the present study, a statistically significant positive relation was found between SOX4 overexpression and the histopathological type of EC with more expression in SEC than EEC. This was compatible with previous studies reporting SOX4 overexpression in small cell lung cancer, triple negative breast cancer and EC, respectively (**26,27 and 28)**. This could be contributed to the role of SOX4 activation of TGF-β and PI3K/Akt pathways that induce tumor progression and aggressiveness.

This disagreed with a study on melanoma reporting higher SOX4 expression in less aggressive histological types **(29)**. This may be contributable to the effect of SOX4 on specific promoters controlling expression of miRNAs through downstream transcription factors as P53 might be an explanation.

In this work, SOX4 overexpression was significantly related to FIGO grade. This may be pursuant to the critical role of SOX4 in promoting tumor progression. In accordance with this finding, similar results were reported in studies on osteosarcoma, EC and hypopharyngeal carcinoma, respectively (**30, 31 and 32)**.

In contrast, A study on EC reported a higher SOX4 expression in G1 than G2/3 EC **(33)**. This may be because of adopting different techniques in their study by using PCR not immunohistochemistry.

Another statistically significant positive relation was reached, between SOX4 overexpression and depth of myometrial invasion of EC. This was referred to its ability to activate of TGF-β and the subsequent induction of numerous SOX4 target genes are involved in cancer cell proliferation, survival, stemness, and EMT resulting in tumor invasion **(6)**. This agreed with studies on gastric carcinoma, EC, and oesophageal squamous cell carcinoma, respectively **(34, 35, 36 and37)**

Despite of previous results, studies on skin and lung tumours, reported that SOX4 overexpression suppressed the invasion of melanoma and lung cancer **(38 and 39)**. This controversy could be due to the intrinsic differences in the biology of different tissue types or the ability of SOX4 to modulate the PUMA (p53 up-regulated modulator of apoptosis) transcription and expression, an inducer of apoptotic cell death, highlighting its role in tumour suppression **(40** **and 41).**

The current study also revealed a significant positive relation between SOX4 overexpression and LVSI. These results could be explained by the acquisition of mesenchymal traits, enhanced cell migration and invasion on SOX4 overexpression **(42)**. This was consistent with studies on laryngeal squamous cell carcinoma, breast cancer and EC, respectively **(43, 44 and 31)**.

This contradicted to a study on hepatocellularcarcinoma noting a significant relation between SOX4 overexpression and LVSI **(45)**. These contradictory results could be attributable to the role of SOX4 in mediating p53 activation resulting in induction of cell cycle arrest and tumor apoptosis.

The current study reached a statistically significant relation between SOX4 overexpression and T/B configuration with more expression in infiltrative than pushing tumor border. There was a statistically significant relation between SOX4 overexpression and T component in the current study. This agreed with studies on oral squamous cell carcinoma, lung and breast cancer, respectively (**46 and 47)**. This could be attributed to SOX4 activation of members of metalloprotease family responsible for the breakdown of the extracellular matrix, amplification of PI3K/AKT pathway and induction of EMT (**48)**.

Overexpression of SOX4 was significantly related to evidence of desmoplasia in studied EC cases. This was pursuant to similar results reported in a study on pancreatic cancer **(49)**. The effect of SOX4 on proliferation and differentiation of cancer associated fibroblasts and myofibroblasts through TGF- β/Smad pathway activation is a proper explanation (**50)**.

Inversely, a previous study on pancreatic ductal adenocarcinoma found that SOX4 can lead to tumour apoptosis and hence decrease desmoplastic reaction **(51)**. This could be explained by that strong activation of EMT by collaboration of TGF-β with oncogenic, hyperactive RAS signalling conflicts with a SOX4-dependent epithelial program, triggering apoptosis (**52)**.

In this work, SOX4 overexpression was significantly related to mitotic count. This agreed with a study on breast cancer **(53)**. This may be because of the possible role of SOX4 activating TGF-β pathway resulting in cellular proliferation.

In contrast, a study on glioblastoma multiforme found that SOX4 overexpression reduced cellular proliferation and mitotic rate **(54)**. This may be due to SOX4 reduction of AKT, results in inhibiting P53 degradation and activation of downstream genes resulting in cell cycle arrest.

This result contradicted to a study of laryngeal squamous cell carcinoma which revealed no relation between SOX4 overexpression and T component **(55)**. This may be contributed to studying larger number of cases and different tumour biology.

This study also noted that SOX4 overexpression was positively related with lymph node metastasis, advanced FIGO and TNM staging of EC. This agreed with studies on breast cancer, colorectal carcinoma and EC, respectively **(56),** **(57 and 31)**. This could be linked to the effect of SOX4 on EMT reducing the mRNA level of the epithelial marker, E-cadherin, and increasing the levels of the three mesenchymal markers, Vimentin, N-cadherin, and Fibronectin (**58)**.

Contrariwise, an inverse relation between SOX4 expression, nodal metastasis and advanced clinical stage, was reported in studies on melanoma and primary gall bladder carcinoma, respectively (**59 and 60)**. This controversy may be due to the role of SOX4 as a DNA damage sensor by blocking p53 degradation; SOX4 promoting cell cycle arrest and apoptosis inhibiting tumorigenesis **(40).**

In the present study, a statistically significant relation between SOX4 overexpression and distant metastasis was reached. This result agreed with previous studies regarding prostate cancer and gastric cancer, respectively **(61and 62)**. Possible activation of C-MYC by SOX4 may play an important role in tumour progression **(37)**.

Inversely, A study on melanoma found that SOX4 overexpression decreased the metastasis **(63)**. Inhibition of tumour initiation through DNA damage signalling, activation of apoptosis and / or downregulation of certain oncogenic pathways as NF-κB signaling pathway were proposed as possible explanations **(64)**.

There was statistically insignificant relation reached between SOX4 overexpression and age of the studied cases being consistent with previous studies on gastric cancer and EC, respectively (**65 and 28)**. Inversely, another study on EC found a significant positive relation between SOX4 overexpression and the age of studied cases **(31)**. This could be explained by studying only EEC cases while the current study was applied to EEC and SEC cases.

The current study reached a statistically significant positive difference between nuclear morphometric parameters in EC and EH. The MNA was increasing along the progression of EH without atypia cases, to AEH to EC (60.37, 73.17 and 134.93 μm2). This could be clarified by that nuclear shape changed from elongated nuclei in EH without atypia to rounded nuclei in AEH and progression to EC by being larger, pleomorphic, and more rounded. This agreed with previous studies on EH and EC (**18 and 7)**.

This study also related the nuclear morphometric parameters with the clinicopathological findings of EC. The MNA showed a statistically significant relation with the type of EC in favour of SEC over EEC. This may be contributed to the higher grade and aggressiveness of SEC with large highly pleomorphic nuclei**~~.~~**

This agreed with previous studies reporting that MNA was related to histopathological type with higher values detected in invasive duct carcinoma, poorly differentiated thyroid carcinoma, SEC, and papillary renal cell carcinoma**,** respectively **(66 ,67**,**68,69 and 70).**

Additionally, this study revealed MNA had statistically significant positive relation with tumour grade and consequently with mitotic count. This agreed with studies on EC and breast cancer, respectively **(71,72 and73)**. This result could be interpreted by that histologic grade includes both cytological and architectural features with nuclear grade has been proved to be more important as stated in WHO classification 5th edition **(4).** Thehigher the tumour grade the more aggressive tumour behaviour is hence increased mitotic count **(74).**

This study revealed a statistically significant positive relation between MNA and T/B configuration with higher value of MNA in tumours with infiltrative than pushing borders and evidence of desmoplasia. This agreed with studies on lung adenocarcinoma and laryngeal carcinoma, respectively **(75 and 76)**. This may be indicative of more aggressive tumour behaviour.

A statistically significant positive correlations were reached between MNA and depth of myometrial invasion, T component, LVSI, lymph node, distant metastasis, FIGO stage and TNM stage in studied EC cases. This agreed with results noted in studies on EC, melanoma, breast cancer, gastrointestinal signet ring carcinoma, and colorectal carcinoma respectively (**77, 78, 79, 80,81and 82)**. These results suggested that tumour with large MNA, and high anaplasia, usually had deeper invasion and metastatic potential and consequently higher stage (**83, 84 and 85)**.

This was in contrary to a previous study on breast cancer revealing that the metastasis was associated with smaller nuclei **(86).** This finding could be due to a higher probability of small nuclei to enter through the wall of small arteries and capillaries and entering between dense collagen fibres.

There was statistically insignificant correlation between age of studied cases and MNA. This was in line with a previous study on EC **(87)**.

Upon correlating the SOX4 expressionand the morphometric parameters in this study, there is a significant statistical correlation between SOX4 and MNA (r=852, P<0.001). To our knowledge, this is the first study to reveal a positive correlation between SOX4 expression score and MNA results in EC.

**Conclusion:**

The significant correlation between SOX4 expression and MNA may imply the pivotal role of SOX4 in cancer development and progression specially in high grade tumours with aggressive behaviour. Also, MNA may distinguish between different tumour grades and types. Hence, our findings suggest that SOX4 and MNA may be used combined as an ancillary diagnostic tool to conventional histopathological findings and predict the prognosis of EC.

**References:**

1. **Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al.** Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2021;71(3):209-49.
2. **Mokhtar N, Salama A, Badawy O, Khorshed E, Mohamed G, Ibrahim M, et al.** Hepatobiliary system tumors, in cancer pathology registry, A 12-year registry 2000-2011. Chapter. 2016;10:161-74.
3. **Yasin HK, Taylor AH, Ayakannu T.** A narrative review of the role of diet and lifestyle factors in the development and prevention of endometrial cancer. Cancers. 2021;13(9):2149**.**
4. **Kim KR, Lax SF, Lazar AJ, Longacre TA, Malpica A, Matias-Guiu X, et al.** Tumours of the uterine corpus. WHO classification of tumours. 5th ed. Female genital tumours. Lyon: International Agency for Research on Cancer. 2020:6.
5. **Levine DA.** Integrated genomic characterization of endometrial carcinoma. Nature. 2013;497(7447):67-73.
6. **Hanieh H, Ahmed EA, Vishnubalaji R, Alajez NM.** SOX4: epigenetic regulation and role in tumorigenesis. Academic Press. InSeminars in cancer biology. 2020; 67: 91-104
7. **KILINÇ AN, FINDIK S, OLTULU P.** Comparative Morphometric Analysis of Nucleus Size in Endometrial Hyperplasia and Carcinoma. Online Türk Sağlık Bilimleri Dergisi. 2020; 5(4):591-601.
8. **Byrd, D. R., Brookland, R. K., Washington, M. K., Gershenwald, J. E., Compton, C. C., et al.***AJCC cancer staging manual* (Vol. 1024). M. B. Amin, S. B. Edge, & F. L. Greene (Eds.). New York: springer 2017.
9. **Amant F, Mirza MR, Koskas M, Creutzberg CL.** Cancer of the corpus uteri. International Journal of Gynecology & Obstetrics. 2018;143: 37-50.
10. **Fuchs Talia L., Angela Chou, Yagiz Aksoy, Mahiar Mahjoub, Amy Sheen, Loretta Sioson, et al.** "A critical assessment of current grading schemes for diffuse pleural mesothelioma with a proposal for a novel mesothelioma weighted grading scheme (MWGS)." *The American Journal of Surgical Pathology*. 2022;46(6): 774-785.
11. S**hakeel Mehroosh, Sajid Mushtaq, Noreen Akhtar, Iftikhar Ali Rana, and Raza Muhammad**. "PATTERNS OF MYOINVASION IN ENDOMETRIOID ADENOCARCINOMA OF ENDOMETRIUM." *Pakistan Armed Forces Medical Journal.* 2021;71, (6): 2211-15.
12. **Sonal Swati, Vikram Deshpande, David T. Ting, Azfar Neyaz, Amaya Pankaj, Martin S. et al.** "Molecular Basis of Tumor Border Configuration in Colorectal Cancer." *Journal of the American College of Surgeons.* 2023;235(5): S15-S16.
13. **Iurchenko, N. P., I. P. Nesina, N. М. Glushchenko, and L. G. Buchynska**. "ROLE OF STROMAL MICROENVIRONMENT IN THE FORMATION OF INVASIVE, ANGIOGENIC, AND METASTATIC POTENTIAL OF ENDOMETRIOID CARCINOMA OF ENDOMETRIUM." *Experimental Oncology.* 2023;45(1): 51-61.
14. **Fang, Chia-Lang, You-Cheng Hseu, Yi-Feng Lin, Shih-Ting Hung, Chein Tai, Yih-Huei Uen, et al.**  "Clinical and prognostic association of transcription factor SOX4 in gastric cancer." *PLoS One* 7, no. 12: e52804, 2012.
15. **Harb Ola A., Amira A. Salem, Rasha Haggag, Shereen El-Shorbagy, and Loay M. Gertallah**. "Immunohistochemical expressions of SQSTM1/p62, Beclin-1, and SOX4 in infiltrating duct carcinoma of the breast." *Egyptian Journal of Pathology* 36, no. 1: 95-103, 2016.
16. **Lu, J. W., M. S. Hsieh, H. A. Hou, C. Y. Chen, H. F. Tien, and L. I. Lin**. "Overexpression of SOX4 correlates with poor prognosis of acute myeloid leukemia and is leukemogenic in zebrafish." Blood Cancer Journal. 2017;7(8): e593-e593.
17. **Shi, Si, Xiaolei Cao, Miao Gu, Bo You, Ying Shan, and Yiwen You**. "Upregulated Expression of SOX4 Is Associated with Tumor Growth and Metastasis in Nasopharyngeal Carcinoma." Age (year).2015;50(14): 5.
18. **AbdRabh, Rasha M., Nehal S. Zafer, Nashwa M. Emara, and Ebtehal M. Abdel Aal**. "Immunohistochemical study of extracellular matrix metalloproteinase inducer expression and nuclear morphometry in endometrial hyperplasia and endometrioid adenocarcinoma." Egyptian Journal of Pathology. 2019;39(1): 141.
19. **Girdhar, Ankita, Kalyani Raju IV, P. N. Sreeramulu, Ankita Girdhar Jr, and Kalyani Raju.** "Significance of Nuclear Morphometry in Breast Lesions: A Cross-Sectional Study." Cureus. 2023;15(5): e39378.
20. **Grimm D, Bauer J, Wise P, Krueger M, Simonsen U, Wehland M, Infanger M, Corydon TJ.** The role of SOX family members in solid tumours and metastasis. InSeminars in Cancer Biology. Academic Press. 2020; 67(Pt. 1): 122-153.
21. **Huang Yi-Wen, Chieh-Ti Kuo, Jo-Hsin Chen, Paul J. Goodfellow, Tim H-M. Huang, Janet S. Rader, and Denise S. Uyar**. "Hypermethylation of miR-203 in endometrial carcinomas." *Gynecologic oncology*. 2014;133(2): 340-345.
22. **Zhao, Xiaomeng, Lili Dai, Qifang Yue, Hua Wang, X. U. Wang, Yuan Li, and Ran Chen**. "MiR-195 inhibits migration, invasion and epithelial-mesenchymal transition (EMT) of endometrial carcinoma cells by targeting SOX4." *Journal of Biosciences*. 2019;44(6): 1-9.
23. **Hu, W. B., L. Wang, X. R. Huang, and F. Li**. "MicroRNA-204 targets SOX4 to inhibit metastasis of lung adenocarcinoma." *European review for medical and pharmacological sciences.* 2019;23(4): 1553-1562.
24. **Chen, Wenhu, Lifang Wang, Xiaoyan Li, Changan Zhao, Liang Shi, Hongguang Zhao, et al.**  "LncRNA SNHG17 regulates cell proliferation and invasion by targeting miR-338-3p/SOX4 axis in esophageal squamous cell carcinoma." *Cell Death & Disease*. 2021;12(9): 806.
25. **Al-Deresawi, Mahdi Saber, Abdul Hussein Moyet AlFaisal, and Salim Rasheed Al-obaidi**. "Role of SOX4 gene in the progression of endometrial hyperplasia to endometrial adenocarcinoma." *Iraqi journal of biotechnology.* 2018;17(2): 82-90.
26. **Castillo, Sandra D., Ander Matheu, Niccolo Mariani, Julian Carretero, Fernando Lopez-Rios, Robin Lovell-Badge, et al** . "Novel transcriptional targets of the SRY-HMG box transcription factor SOX4 link its expression to the development of small cell lung cancer." *Cancer research*. 2012;72(1): 176-186.
27. **Mehta, Gaurav A., Joel S. Parker, Grace O. Silva, Katherine A. Hoadley, Charles M. Perou, and Michael L. Gatza**. "Amplification of SOX4 promotes PI3K/Akt signaling in human breast cancer." *Breast cancer research and treatment* 162, no. 3: 439-450, 2017.
28. **Liao, Hongli, and Peng Lin**. "Expression of SOX4 in Endometrial Cancer and its Relationship with Clinical-pathological Characteristics in Endometrial Cancer Patients." *Journal of Clinical and Nursing Research.* 2020;4(4)
29. **Li, Jun, Zhizhong Zhang, and Gang Li**. "Patient outcome prediction using multiple biomarkers in human melanoma: A clinicopathological study of 118 cases." *Experimental and therapeutic medicine* 2, no. 1: 131-135, 2011.
30. **Chen, Dong, Chuanzhen Hu, Gen Wen, Qingcheng Yang, Changqing Zhang, and Huilin Yang**. "Downregulated sox4 expression suppresses cell proliferation, migration, and induces apoptosis in osteosarcoma in vitro and in vivo." *Calcified Tissue International*. 2018;102(1): 117-127.
31. **Atwa, Hanaa A., Amira Elwan, and Nehal S**. Abouhashem. "Clinicopathological Features, Prognostic Impact and Treatment Outcome of EDIL3 and SOX4 Expressions in Endometrial Adenocarcinoma." *Middle East Journal of Cancer.* 2023;14(1): 82-91.
32. **Zhang, Xiao-bo, Hui-ting Liu, Yuan-ru Liu, Fan Zuo, Zhi-xia Wang, Qian-qian Ren, Yao Yao et al**. "Overexpression of SOX4 indicates poor prognosis and promotes progression of hypopharyngeal carcinoma.". 2023.
33. **Saegusa, Makoto, Miki Hashimura, and Takeshi Kuwata**. "Sox4 functions as a positive regulator of β-catenin signaling through upregulation of TCF4 during morular differentiation of endometrial carcinomas." *Laboratory investigation.* 2012;92(4): 511-521.
34. **Ding, Lixian, Yuying Zhao, Shuwei Dang, Yue Wang, Xinglong Li, Xiaotong Yu, et al.** "Circular RNA circ-DONSON facilitates gastric cancer growth and invasion via NURF complex dependent activation of transcription factor SOX4." *Molecular cancer*. 2019;18(1): 1-11.
35. **Wang, Lei, Shanshan Zhao, and Y. U. Mingxin**. "LncRNA NR2F1-AS1 is involved in the progression of endometrial cancer by sponging miR-363 to target SOX4." *Die Pharmazie-An International Journal of Pharmaceutical Sciences.* 2019;74(5): 295-300.
36. **HUANG, LIU Jia-shu FAN Bo, and Jin LIU Sheng-feng**. "Expression of SOX4 gene and its biological effects in endometrial carcinoma." *Acta Anatomica Sinica.* 2021;52(3): 425.
37. **Wu, Zilong, Boyao Yu, and Lei Jiang**. "MiR-212-3p mediates apoptosis and invasion of esophageal squamous cell carcinoma through inhibition of the Wnt/β-catenin signaling pathway by targeting SOX4." *Journal of Thoracic Disease*. 2020;12(8): 4357.
38. **Jafarnejad, Seyed Mehdi, Aijaz Ahmad Wani, Magdalena Martinka, and Gang Li**. "Prognostic significance of Sox4 expression in human cutaneous melanoma and its role in cell migration and invasion." *The American journal of pathology.* 2010;177(6): 2741-2752.
39. **Jang Sang-Min, Eun-Jin Kang, Jung-Woong Kim, Chul-Hong Kim, Joo-Hee An, and Kyung-Hee Choi**. "Transcription factor Sox4 is required for PUMA-mediated apoptosis induced by histone deacetylase inhibitor, TSA." *Biochemical and biophysical research communications.* 2013;438(2): 445-451.
40. **Pan, Xin, Jie Zhao, Wei-Na Zhang, Hui-Yan Li, Rui Mu, Tao Zhou, et al**. "Induction of SOX4 by DNA damage is critical for p53 stabilization and function." *Proceedings of the National Academy of Sciences*. 2009;106(10): 3788-3793.
41. **Miyazaki Makoto, Ryo Otomo, Yuko Matsushima-Hibiya, Hidenobu Suzuki, Ayana Nakajima, Naomi Abe, et al**. "The p53 activator overcomes resistance to ALK inhibitors by regulating p53-target selectivity in ALK-driven neuroblastomas." *Cell Death Discovery.* 2018; 4, (1): 56.
42. **Bagati, Archis, Sushil Kumar, Peng Jiang, Jason Pyrdol, Angela E. Zou, Anze Godicelj, et al**. "Integrin αvβ6–TGFβ–SOX4 pathway drives immune evasion in triple-negative breast cancer." *Cancer cell*. 2021;39(1): 54-67.
43. **Franz, Leonardo, Lorenzo Nicolè, Anna Chiara Frigo, Giancarlo Ottaviano, Piergiorgio Gaudioso, Tommaso Saccardo, et al**. "Epithelial-to-mesenchymal transition and neoangiogenesis in laryngeal squamous cell carcinoma." *Cancers*. 2021;13(13): 3339.
44. **Fan, Shan‐Ji, Ying Cui, Yue‐Hua Li, Jia‐Chi Xu, Ying‐Ying Shen, Hong Huang, et al**. "LncRNA CASC9 activated by STAT3 promotes the invasion of breast cancer and the formation of lymphatic vessels by enhancing H3K27ac‐activated SOX4." *The Kaohsiung Journal of Medical Sciences*. 2022;38(9): 848-857.
45. **Hur, Wonhee, Hyangshuk Rhim, Chan Kwon Jung, Jin Dong Kim, Si Hyun Bae, Jeong Won Jang, et al**. "SOX4 overexpression regulates the p53-mediated apoptosis in hepatocellular carcinoma: clinical implication and functional analysis in vitro." *Carcinogenesis*. 2010;31(7): 1298-1307.
46. **Watanabe, Masahiro, Yuichi Ohnishi, Masahiro Wato, Akio Tanaka, and Kenji Kakudo**. "SOX4 expression is closely associated with differentiation and lymph node metastasis in oral squamous cell carcinoma." *Medical Molecular Morphology*. 2014;3(47): 150-155.
47. **Sasaki, Aya, Hitoshi Abe, Satsuki Mochizuki, Masayuki Shimoda, and Yasunori Okada**. "SOX4, an epithelial–mesenchymal transition inducer, transactivates ADAM28 gene expression and co‐localizes with ADAM28 at the invasive front of human breast and lung carcinomas." *Pathology international*. 2018;68(8): 449-458.
48. **Mehta, Gaurav A., Joel S. Parker, Grace O. Silva, Katherine A. Hoadley, Charles M. Perou, and Michael L. Gatza**. "Amplification of SOX4 promotes PI3K/Akt signaling in human breast cancer." *Breast cancer research and treatment*. 2017;162(3): 439-450.
49. **Luo, Qiang, Ziquan Hu, Hongcheng Zhao, Yanmei Fan, Xiancong Tu, Yueqing Wang, et al.** "The role of TGF-β in the tumor microenvironment of pancreatic cancer." *Genes & Diseases*. 2023;10(4): 1513-1524.
50. **Bulle, Ashenafi, and Kian-Huat Lim**. "Beyond just a tight fortress: contribution of stroma to epithelial-mesenchymal transition in pancreatic cancer." *Signal Transduction and Targeted Therapy.* 2020; 5(1): 249.
51. **David, Charles J., Yun-Han Huang, Mo Chen, Jie Su, Yilong Zou, Nabeel Bardeesy, et al.**. "TGF-β tumor suppression through a lethal EMT." *Cell.* 2016; 164(5): 1015-1030.
52. **Lee, Jun Ho, and Joan Massagué**. "TGF-β in developmental and fibrogenic EMTs." In *Seminars in cancer biology*. Academic Press. 2022;86 (Pt 2):136-145.
53. **Vervoort, Stephin J., Olivier G. de Jong, M. Guy Roukens, Cynthia L. Frederiks, Jeroen F. Vermeulen, Ana Rita Lourenço, et al**. "Global transcriptional analysis identifies a novel role for SOX4 in tumor-induced angiogenesis." *Elife*. 2018;7: e27706.
54. **Zhang, Jing, Huawei Jiang, Jiaofang Shao, Ruifang Mao, Jie Liu, Yingying Ma, et al.**  "SOX4 inhibits GBM cell growth and induces G0/G1 cell cycle arrest through Akt-p53 axis." *BMC neurology* 2014;14(1): 1-11.
55. **Zhang, Mingjie, Hui Li, Yuefeng Han, Mengjun Wang, Junjie Zhang, and Shiyin Ma**. "Clinicopathological significance of SOX4 and epithelial-mesenchymal transition markers in patients with laryngeal squamous cell carcinoma." *Auris Nasus Larynx*. 2021;48(6): 1167-1175.
56. **Zhang, Jing, Chunhua Xiao, Zhenbo Feng, Yun Gong, Baohua Sun, Zhongqi Li, et al**. "SOX4 promotes the growth and metastasis of breast cancer." *Cancer cell international*. 2020;20: 1-11.
57. **Pan, Shuang, Dongyan Bao, Yao Li, Dahua Liu, Shuai Quan, and Rong Wang**. "SOX4 induces drug resistance of colorectal cancer cells by downregulating CYLD through transcriptional activation of microRNA‐17." *Journal of Biochemical and Molecular Toxicology*. 2022;36(1): e22910.
58. **Liu, Yu, and Wenjun Guo**. "SOX factors as cell-state regulators in the mammary gland and breast cancer." In *Seminars in cell & developmental biology*. Academic Press. 2021;114: 126-133.
59. **Li, Jun, Zhizhong Zhang, and Gang Li**. "Patient outcome prediction using multiple biomarkers in human melanoma: A clinicopathological study of 118 cases." *Experimental and therapeutic medicine*. 2011;2(1): 131-135.
60. **Wang, Chengguo, Huadong Zhao, Jianguo Lu, Jikai Yin, Li Zang, Nuan Song, et al**. "Clinicopathological significance of SOX4 expression in primary gallbladder carcinoma." *Diagnostic Pathology*. 2012;7(1): 1-7.
61. **Liu, Hui, Zhen Wu, Haibin Zhou, Wenjie Cai, Xinjun Li, Jing Hu, et al**. "The SOX4/miR-17-92/RB1 axis promotes prostate cancer progression." *Neoplasia*. 2019;21(8): 765-776.
62. **Zhang, Jianfeng, Kai Zhang, and Yingkui Hou**. "Long non‑coding RNA NNT‑AS1 knockdown represses the progression of gastric cancer via modulating the miR‑142‑5p/SOX4/Wnt/β‑catenin signaling pathway." *Molecular Medicine Reports*. 2020;22(2): 687-696.
63. **Jafarnejad, Seyed Mehdi, Gholamreza Safaee Ardekani, Mazyar Ghaffari, Magdalena Martinka, and Gang Li**. "Sox4-mediated Dicer expression is critical for suppression of melanoma cell invasion." *Oncogene*. 2013;32(17): 2131-2139.
64. **Geethadevi, Anjali, Ansul Sharma, Manish Sharma, and Deepak Parashar**. "An interplay between MicroRNA and SOX4 in the regulation of epithelial-mesenchymal transition and cancer progression." *Cancer Translational Medicine.* 2018; 4(1):17-17.
65. **Yuan, Xiao, Shuanhu Wang, Mulin Liu, Zhen Lu, Yanqing Zhan, Wenbin Wang, et al.** "Histological and pathological assessment of miR-204 and SOX4 levels in gastric cancer patients." *BioMed Research International.* 2017;2017: 6894675-6894675.
66. **Abdalla, Fathi, Jamela Boder, Rabia Markus, Hussein Hashmi, Abdelbaset Buhmeida, and Yrjö Collan**. "Correlation of nuclear morphometry of breast cancer in histological sections with clinicopathological features and prognosis." *Anticancer research*. 2009;29(5): 1771-1776.
67. **AbdelMaksoud, Ahlam A., Ghada A. AbdElfattah, Ranih Z. Amer, and Nashwa M. Emara**. "Evaluation of galectin-3, β-catenin, and nuclear morphometry in different thyroid lesions." *Egyptian Journal of Pathology*. 2013;33(2): 129-138.
68. **Yamaguchi, T., A. Kawahara, S. Hattori, T. Taira, H. Abe, S. Sanada, J. Akiba et al**. "Cytological nuclear atypia classification can predict prognosis in patients with endometrial cancer." *Cytopathology*. 2015;26(3): 157-166.
69. **Norimatsu, Yoshiaki, Satoshi Irino, Yoshinobu Maeda, Kenji Yanoh, Tetsuji Kurokawa, Yasuo Hirai, et al.** "Nuclear morphometry as an adjunct to cytopathologic examination of endometrial brushings on LBC samples: a prospective approach to combined evaluation in endometrial neoplasms and look alikes." *Cytopathology*. 2021;32(1): 65-74.
70. **Agrawal, Shruti, and Nikunj Jain**. "Nuclear Morphometry is a superior Prognostic Predictor in comparison to Histological grading in Renal cell Carcinoma: A Retrospective Clinico-pathological study." *medRxiv.* 2021: 2021-01.
71. **Mahovlić, Vesna, Ana Ovanin-Rakić, Lada Škopljanac-Mačina, Ana Barišić, Sanda Rajhvajn, Danijela Jurič, et al**. "Digital morphometry of cytologic aspirate endometrial samples." *Collegium antropologicum*. 2010;34(1): 45-51.
72. **Gupta, P., N. Gupta, and P. Dey**. "Morphometric analysis of endometrial cells in liquid‐based cervical cytology samples." *Cytopathology*. 2017;28(2): 140-148.
73. **Kalhan, Shivani, Shilpa Garg, Rahul Satarkar, Puja Sharma, Sonia Hasija, and Sonia Sharma**. "Correlation of nuclear morphometry with clinicopathologic parameters in malignant breast aspirates." *South Asian journal of cancer*. 2021;10(02): 64-68.
74. **Kesarkar, Kashmira, Avinash Tamgadge, Treville Peirera, Sandhya Tamgadge, Swati Gotmare, and Pooja Kamat**. "Evaluation of mitotic figures and cellular and nuclear morphometry of various histopathological grades of oral squamous cell carcinoma: comparative study using crystal violet and Feulgen stains." *Sultan Qaboos University Medical Journal*. 2018;18(2): e149.
75. **Canzonieri, V., S. Monfardini, and A. Carbone**. "Defining prognostic factors in malignancies through image analysis." *European Journal of Cancer*. 1998;34(4): 451-458.
76. **Xu, Lauren, Fabio Tavora, and Allen Burke**. "Histologic features associated with metastatic potential in invasive adenocarcinomas of the lung." *The American journal of surgical pathology*. 2013;37(7): 1100-1108.
77. **Ørbo, A., M. Rydningen, B. Straume, and S. Lysne**. "Significance of morphometric, DNA cytometric features, and other prognostic markers on survival of endometrial cancer patients in northern Norway." *International Journal of Gynecologic Cancer*. 2002;12(1): 49-56.
78. **Mutter, George L., James Kauderer, Jan PA Baak, and David Alberts**. "Biopsy histomorphometry predicts uterine myoinvasion by endometrial carcinoma: a Gynecologic Oncology Group study." *Human pathology*. 2008;39(6): 866-874.
79. **Nunes, Tatiana WN, Eduardo C. Filippi-Chiela, Sidia M. Callegari-Jacques, Vinicius D. da Silva, Tatiana Sansonowicz, Guido Lenz, et al.**. "Nuclear morphometric analysis in tissue as an objective tool with potential use to improve melanoma staging." *Melanoma Research*. 2019;29(5): 474-482.
80. **Kalhan, Shivani, Shilpa Garg, Rahul Satarkar, Puja Sharma, Sonia Hasija, and Sonia Sharma**. "Correlation of nuclear morphometry with clinicopathologic parameters in malignant breast aspirates." *South Asian journal of cancer*. 2021;10(02): 64-68.
81. **Da, Qian, Shijie Deng, Jiahui Li, Hongmei Yi, Xiaodi Huang, Xiaoqun Yang, et al**. "Quantifying the cell morphology and predicting biological behavior of signet ring cell carcinoma using deep learning." *Scientific Reports*. 2022;12(1): 183.
82. **Kumar, Neeraj, Ruchika Verma, Chuheng Chen, Cheng Lu, Pingfu Fu, Joseph Willis, et al**. "Computer‐extracted features of nuclear morphology in hematoxylin and eosin images distinguish s tage II and IV colon tumors." *The Journal of pathology*. 2022;257(1): 17-28.
83. **Ji, Meng-Yao, Lei Yuan, Xiao-Da Jiang, Zhi Zeng, Na Zhan, Ping-Xiao Huang, et al.** "Nuclear shape, architecture and orientation features from H&E images are able to predict recurrence in node-negative gastric adenocarcinoma." *Journal of translational medicine*. 2019;17(1): 1-12.
84. **WU, Boda, Meijun LONG, and Mei YANG**. "Correlation of nuclear morphometry with clinicopathological features in breast cancer." *Journal of Chinese Physician.* 2020: 1316-1321.
85. **Tortorella, Lucia, Stefano Restaino, Gian Franco Zannoni, Giuseppe Vizzielli, Vito Chiantera, Serena Cappuccio, et al**. "Substantial lymph-vascular space invasion (LVSI) as predictor of distant relapse and poor prognosis in low-risk early-stage endometrial cancer." *Journal of gynecologic oncology*. 2021;32(2): 11-11.
86. **Duca-Barbu, Simona-Alina, Alexandru Adrian Bratei, Antonia-Carmen Lisievici, Tiberiu Augustin Georgescu, Bianca Mihaela Nemes, et al.**. "A Novel Algorithm for Evaluating Bone Metastatic Potential of Breast Cancer through Morphometry and Computational Mathematics." *Diagnostics.* 2023;13(21): 3338.
87. **Salvesen, Helga B., Ole Erik Iversen, and Lars A. Akslen**. "Prognostic impact of morphometric nuclear grade of endometrial carcinoma." *Cancer: Interdisciplinary International Journal of the American Cancer Society*. 1998;83(5): 956-964.