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Expression of NKX 3.1 in Sertoli Cell Tumors

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ABSTRACT

Objective: To evaluate the NKX3.1 expression by immunohistochemistry in normal testicular parenchyma and in Sertoli cell tumours and Sertoli Leydig cell tumours of the testes and ovary.

Study Design: Retrospective longitudinal study.

Place and Duration of Study: Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore Pakistan, from 2010-2021.

Methodology: We used immunohistochemistry to evaluate the positivity and loss of nuclear expression of NKX3.1 in the Sertoli cell tumour (11 cases), Sertoli Leydig cell tumour (31 cases) and in normal testicular parenchyma (7 cases).

Results: In our study, there were 49 cases. All the cases of benign testicular parenchyma expressed positivity with nuclear staining of NKX 3.1 in Sertoli cells. Two out of 11 Sertoli cell tumours expressed positivity with nuclear positivity of NKX 3.1 in Sertoli cell component (18.18%) and 9 of the cases showed loss of staining of NKX 3.1 (81.8%). All Sertoli Leydig cell tumours showed loss of staining of NKX 3.1.

Conclusion: Nuclear expression of NKX 3.1 is seen in Sertoli cells of normal testicular parenchyma. This staining is lost in Sertoli cell tumours and Sertoli Leydig cell tumours.

Keywords: NKX 3.1 loss, Sertoli cell tumour, Sertoli-leydig cell tumor.

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INTRODUCTION

Sertoli Leydig cell tumours are rare (<0.5% of ovarian neoplasms). Most of the tumours present at a young age. Patients present with virilization or iso-sexual precocity. Tumours can be solid (fleshy pale yellow) or cystic with a mean size of 12-14cm.[1] These tumours are divided into six categories. The basis for this division is differentiation and heterologous elements.[2] The categories are well differentiated(11%), moderately differentiated (54%), with poor differentiation (13%), tumours with heterologous elements, tumours with retiform pattern and pure Sertoli cell tumors.[3,4]

Sertoli cell tumours are mostly unilateral, have bland cytology and are associated with peutz jeghers syndrome. The size varies from 4 to 12cm (mean size 8cm). They are usually solid (colour tan to yellow) but can be cystic.[5,6] Sertoli cell tumours present mostly in a tubular pattern. Less common patterns include diffuse, corded, trabecular, pseudopapillary, retiform, and spindled patterns.[7]

There are many potential mimickers of Sertoli cell tumours, i.e. carcinoid, endometroid adenocarcinoma and well-differentiated Sertoli Leydig cell tumours.[8] Sertoli cell tumours show expression of inhibin, calretinin, WT1, SF1, keratin and SOX9 by immunohistochemistry, and there is negativity for EMA, PLAP, and CEA.

NKX 3.1 is present on chromosome.[8,9] It is a transcription factor that plays an important role in prostate development. It undergoes loss of heterozygosity during the formation of prostate cancer. NKX 3.1 increases the stability of p53.[10] Uptill; much less is known about NKX 3.1 expression by immunohistochemistry in testicular tissue and germ cell tumours (testicular and ovarian). Therefore, we evaluated the NKX 3.1 expression in normal testes parenchyma, Sertoli-cell tumours and Sertoli-Leydig cell tumours.

METHODOLOGY

This retrospective longitudinal study was conducted at Shaukat Khanum Memorial Cancer Hospital, Lahore from 2010 to 2021. Forty-nine cases were retrospectively obtained. The study was approved by the Institutional Review Board of the Shaukat Khanum Memorial Cancer Hospital (IRB number EX-18-05-20-02). Samples were obtained retrospectively from surgically resected specimens previously fixed in formalin and embedded in paraffin.

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Inclusion Criteria: All patients with Sertoli cell tumours and Sertoli Leydig cell tumours from 2010 to 2021 were included in the study.

Exclusion Criteria: Patients with other sex cord-stromal and germ cell tumours were excluded from the study.

Slides were reviewed, and a representative block from each case was then selected. The characteristics, i.e. age of the patient, type of tumour, site, size and focality of the tumour, grade of tumour and presence or absence of lymph node and distant metastasis, were collected. Sections were cut from the blocks and stained using BOND ER2 and ready-to-use monoclonal rabbit antibody. Clone: EP 356 was used. The manufacturer's protocol was followed. Retrieval of the antigen was done for 40 minutes at 100°C temperature. The incubation time of the antibody was 15 minutes. The tissues were 3-5 microns thick. Controls (positive and negative) were applied. The level of NKX 3.1 was observed by the percentage of nuclear staining in tumour cells. Staining intensity was graded at three levels, i.e. weak, moderate or severe. The extent of staining was also scored at three levels, i.e. negative, focal positive and diffuse positive. Staining in 0 or <5% of cells was graded as negative, staining in 5 to 50% of cells was graded as focal positive and staining in >50% of cells was graded as diffuse positive.[11,12]

NKX 3.1 was applied by immunohistochemistry on all these 49 cases. The level of staining of NKX 3.1 was observed by the percentage of nuclear staining seen in tumour cells. Staining intensity was graded at three levels, i.e. weak, moderate or severe. The extent of staining was also scored at three levels, i.e. negative, focal positive and diffuse positive. Staining in 0 or <5% of cells was graded as negative, staining in 5 to 50% of cells was graded as focal positive and staining in >50% of cells was graded as diffuse positive.[13]

The statistical analysis was performed using SPSS (version 20). Mean and standard deviation was calculated for quantitative variables. Frequency and percentage were calculated for qualitative variables.

RESULTS

In our study, there were 49 cases. Eleven cases (22.4%) were of Sertoli cell tumours (Figure-A), 31 cases (63.2%) were of Sertoli Leydig cell tumours (Figure-B and C), and 7 cases (14.2%) were of normal testicular parenchyma. In addition, 3 of the Sertoli-Leydig cell tumours also showed normal testicular parenchyma at the periphery. 4(12.9%) of the Sertoli Leydig cell tumours showed heterologous elements (Figure-D), and 4(12.9%) showed a retiform pattern.

Thirty-eight patients were females, and 11 patients were males. The age range of the patients was 9 to 67 years (mean age: 31.84±15.64 years). The size of the tumour ranged from 3 to 28cm, with a mean size of 12.45±6.72cm.

2 out of 11 cases of Sertoli cell tumours showed positive nuclear expression of NKX3.1 (Figure-E) in tumour cells (18.18%). All cases of benign testicular parenchyma showed diffuse nuclear positivity of NKX 3.1 in Sertoli cells (Figure-F).

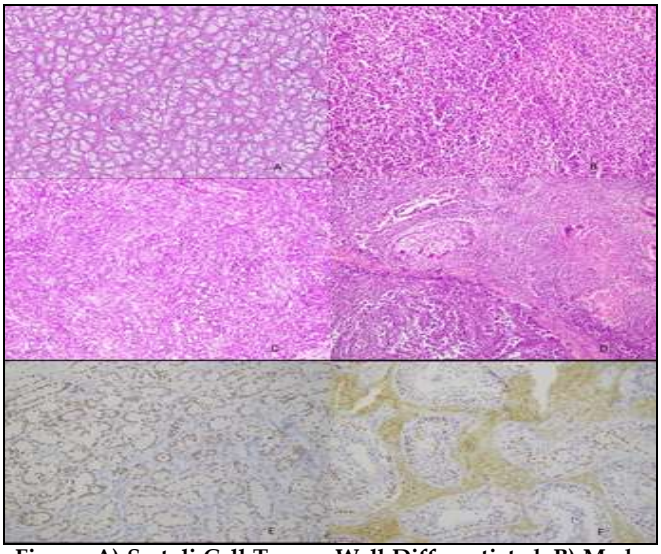

Figure: A) Sertoli Cell Tumor, Well Differentiated; B) Moderately Differentiated Sertoli-Leydig Cell Tumor; C) Poorly Differentiated Sertoli-Leydig Cell Tumor; D) Sertoli-Leydig Cell Tumor With Heterologous Elements E) NKX3.1 staining in Well Differentiated Sertoli Cell Tumor F) NKX3.1 staining in Sertoli Cells of seminiferous tubules

One of the tumours showing positive staining for NKX 3.1 was a well Differentiated Sertoli Leydig Cell tumour (diffuse staining in >50% of cells). The other case showing NKX3.1 positivity was a large cell calcifying Sertoli cell tumour with focal NKX 3.1 expression (nuclear staining in 40% of cells). Nine of the cases of Sertoli cell tumours showed loss of staining of NKX3.1 (81.8%) (Table-I). All Sertoli-Leydig cell tumours showed loss of staining of NKX 3.1.

Table-I: Number of Cases included in the study (n=49)

Diagnosis	n(%)
Sertoli cell tumor	11(22.4%)
Sertoli-Leydig cell tumor	31(63.2%)
Sertoli-Leydig cell tumor with heterologous elements	3(6.2%)
Sertoli-Leydig cell tumor with retiform pattern	4(8.2%)

Ten tumours were fragmented, so their exact size could not be determined. Thirty-nine tumours showed unilaterality, and three tumours showed bilaterality. Eight tumours were well differentiated, 21 were moderately differentiated, 12 were poorly differentiated, and one was a metastatic tumour in the inguinal lymph node (Table II).

Table-II: Differentiation of Tumors (n=42)

Differentiation	n(%)
Well differentiated	8(19.1%)
Moderately differentiated	21(50.0%)
Poorly differentiated	12(28.6%)
Metastatic	1(2.3%)

DISCUSSION

Sex cord-stromal tumours occur in adolescents and young adults (First 20-30 years of life). They can present as a pelvic mass and can have hormonal manifestations. Sertoli Leydig cell tumours comprise 20% of sex cord-stromal tumours. These tumours can have well differentiation (delicate stroma separates Sertoli cell tubules, and this stroma contains Leydig cells), can have intermediate differentiation (lobules, cords and tubules of Sertoli cells surrounded by stromal Leydig cells) or poor differentiation(usually sarcomatoid areas). Heterologous elements can be present and include mucinous epithelial glands & rhadomyosarcomatous or chondrosarcomatous elements.[14] Sertoli Leydig cell tumours express positive staining of inhibin, calretinin, WT1 and CD99.[15]

NKX 3.1 is present on chromosome.[8] It is a gene which expresses itself in the testes, and prostate NKX 3.1 loss is associated with prostate development and carcinogenesis. Until now, very few studies have explored NKX 3.1 expression in stromal tumours of the testes and ovaries. In addition, very little is known about the role of NKX 3.1 in normal testicular parenchyma and testicular and ovarian sex-cord stromal tumours. In our study, the loss of NKX 3.1 is seen in 81.81% of Sertoli cell tumours and 100% of Sertoli Leydig cell tumours. In a study by Skotheim *et al.*[16] diffuse staining for NKX3.1 was seen in normal testicular germ cells (17 out of 17 cases) and carcinoma in situ (17 out of 17 cases).

In a study by Arnesen *et al*. NKX 3.1 immunohistochemical stain's diffuse staining was seen in Sertoli cells of normal testicular parenchyma (12 out of 12 cases). In that study, the positivity of NKX 3.1 by immunohistochemistry was seen in 2 out of 22 sex-cord stromal tumours. One of the tumours was a Sertoli-Leydig cell, well-differentiated (5% of tumour cells showed focal staining), and the other was a large calcifying cell Sertoli cell tumour (diffuse moderate staining in nuclei). No staining was seen in Leydig cells and rete testes epithelium.[17]

In a study by Gelmann *et al.*[18] 4061 human tissues were analyzed for staining with NKX 3.1. The results show that NKX 3.1 staining was present in normal testicular tissue (57% positivity). However, they needed to provide details about the cells showing a positive staining pattern. They did not find any positive staining in testicular Leydig and germ-cell tumours. No Sertoli-cell tumours or Sertoli-Leydig cell tumours were included in their study.[18]

Our study showed that NKX 3.1 is expressed in Sertoli cells of normal seminiferous tubules of the testes. However, its expression is downregulated in Sertoli cell tumours and Sertoli-Leydig cell tumours of the testes and ovary. Therefore, the loss of NKX 3.1 is associated with the process of tumour formation. Previous studies also support our study as they also showed that NKX 3.1 loss is necessary for the normal function of the testes, and its negativity is associated with invasive tumours of testicular origin.

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CONCLUSION

We conclude that the nuclear expression of NKX 3.1 is seen in normal testicular parenchyma. Furthermore, this expression is seen in Sertoli cells of seminiferous tubules in normal testes. However, the loss of this staining is seen in Sertoli cell tumours and Sertoli Leydig cell tumours. This feature can help differentiate Sertoli cell tumours from normal seminiferous tubules, benign proliferations of Sertoli cells and metastatic carcinoma of prostatic origin.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

AF: Conception, drafting the manuscript, approval of the final version to be published.

SM: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

AL: Data acquisition, data analysis, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity

of any part of the work are appropriately investigated and resolved.

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