Research Article

The Immunohistocehmical Expression of Beta Human Chorionic Gonadotrophin (β-HCG) in Different Grades of Human Papillary Urothelial Neoplasm

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Abstract

Background: Urothelial carcinomais the most common urological malignancy and following prostate and lung cancer, it is the third most common carcinoma in male population

Objective: The main aim of this study was to determine the expression of beta hCG in different grades of human urothelial carcinoma in our population.

Methods: The study was conducted in Pathology Department of Faisalabad Medical University, Faisalabad, between 2018-2020.Sections were taken on frosted slides for H& E, and on lysine coated slides for IHC.H& E and IHC were performed according to protocols.

Results: Out of the 65 tumor studied, 35(54%) were graded as low and 30(46%) were graded as high. In 35 low grade tumors 4(11%) were muscle invasive and 31(89%) were non-invasive and in 30 high grade tumors, 24(80%) were found to be muscle invasive and only 6(20) were non muscle invasive. Out of 65 specimens, 52 (80%) cases were found to be positive for beta hCG while only 13(20%) were found to be negative of 35 low grade tumors, 27(77%) tumors were stained positive for beta hCG and 8(23%) were negative. Of 30 high grade tumors 25(83%) tumors were stained positive for beta hCG and 5 (17%) were negative. Chi-square test was applied showed that there was no significant association between tumor grade and beta hCG expression.

Conclusion: The immunohistochemical expression of beta hCG is positive in majority of low and high grade urothelial carcinoma but it may not be utilized as a marker for prognostic determination.

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Keywords | Urothelial carcinoma, Beta hCG, muscle invasion.

Introduction

Carcinoma of urinary bladder is ranked at number 10 among the most common carcinomas in the world with an estimated 54,9000 newly reported cases. Of which 200,000 died of this disease.[1] Urothelial carcinoma is important with regard to the high medical costs and its impact on the patient's quality of life. Although its treatment costs exceed all other types of cancer, these treatments extend the time to recurrence, not the survival . Urothelial carcinoma usually affects people belonging to older age group. The estimated age group at diagnosis is 69 years in males and in females it is 71 years.[2] Males are 2.5 to 4 times more prone of

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developing urothelial carcinoma than females.[3] Beta human chorionic gonadotropin, was the first hormone to be declared as 'Pregnancy Hormone'by Ascheim and Zondeik in 1928. It comprises of two subunits, Alpha subunit, which is specific for the glycoprotein proteins family and β-subunit- specific for receptors.[4] The β-subunit of hCG, is also produced by many non-trophoblastic cancers like vaginal carcinoma, colorectal carcinoma, breast cancer, bladder cancer, pulmonary tumors, colorectal carcinoma, prostatic carcinoma and gastrointestinal cancers. Thus, β-hCG exerts carcinogenic effects by inhibiting the apoptotic function of Transforming growth factor β-1 in tumor cells of various origin by binding with TGFβ receptor. Expression of beta hCG by urothelial carcinoma is closely linked with a tumor of higher grade and poor prognosis. hCG beta positive tumors are radio-resistant and have more metastatic potential than hCG negative tumors.[5]

Development of a novel antibody-based dendritic cell (DC)-focussed cancer vaccines are capable of initiating the cellular immune responses directed towards β-hCG. Thus DC-targeted hCG-beta vaccines hold a promising result for the control of a number of cancers and provides further clinical development.[6] Hence my study focuses on the expression of beta hCG receptors in bladder cancer patients presenting in our local population and its association among different grades. In the light of literature, it has been observed that the high grade tumors presents with higher expression of beta hCG so immunohistochemical expression of this marker would likely predict the superficial tumors that would recur and high grade lesions with inferior outcome.

Methods

This retrospective study was conducted in Department of Pathology, Faisalabad Medical University. Blocks and relevant information was retrieved form record room of the department. Non-probability, Purposive sampling technique was used.

The sample size was derived using the following formulae, as per WHO "Sample size determination in Health studies" software.

$$n = \frac{Z^2 \times P(1-P)}{d^2}$$

here

Z = 3.84 at 95% confidence level., P = Anticipated Sensitivity (0.214 %) [7]

d = Desired precision = 0.1 %.

n = Sample size. Required sample size is 65.

Total 65 cases of urothelial carcinoma were obtained. All histologically diagnosed cases of papillary urothelial carcinoma and patients of all ages and gender were included in the study. The patients who have undergone chemotherapy, radiotherapy and those with history of chronic debilitating illness and with co-morbidites (uncontrolled diabetes, HIV, hypertension) were excluded from the study. Formalin fixed paraffin embedded blocks were taken and tissue was obtained on albuminized slide for H&E staining and other on poly-L lysine coated slide for immunohistochemistry. For positive control sections from placenta were taken and processed according to standard protocols. Staining was done as per protocols and manufacturer instructions. Tumor was graded according to simplified WHO/ISUP classification.[8] Immunostained slides were examined and scoring was done using following criteria.

The result of β-hCG immunostaining was based on cytoplasmic staining. Staining intensity was recorded from 0-3 as negative(0), weak(1), moderate(2), strong(3) and percentage of positive cells were evaluated as (0-4), negative(0), >25% (1), 27-50%(2), 51-75%(3), more than 75. Final scoring was done by multiplying the intensity (I) of staining by the percentage of positive cell (P) and the staining expression was represented as Low expression (1-3), Intermediate expression (4-5) High expression (6-7) In the final scrutiny of data all the urothelial tumours which are expressing weak, moderate or strong staining were labelled as a positive case and those showing no staining (zero) were marked as negative.[9]

Results:

Majority of the patients 47 (72%) were above the 50 years of age, 13 patients (20%) were in fifth decade and only 5 patients (8%) were below 40 years. Male patients were 55(85%) and female patients were only 10(15%). Male to female ratio calculated in this study was 5.6: 1. The histological examination of the cases of urothelial carcinoma showed that there were 35 (54%) low grade tumors and 30 (46%) were high grade tumors of 65 cases of urothelial carcinoma 37(57%) showed no muscle

invasion and 28(43%) have muscle invasion. In 35 low grade urothelial carcinoma cases 4(11%) showed muscle invasion while 31/35(89%) of low grade tumors were non-invasive. Of high grade urothelial carcinoma cases 6/30 (20%) were non-invasive and 24/30 showed muscle invasion. The immnunohistochemical analysis of the cases showed 52 (80%) were stained positive for beta hCG and only 13 (20%) were negative, (Table 1). In Low grade urothelial carcinoma cases 27(77%) showed positivity for beta hCG and only 8/35 (23%) were stained negative. 25/30 (83%) of high grade urothelial carcinoma cases were stained positive for beta hCG and only 5/30(17%) showed negative staining (Table 2). In non-muscle invasive urothelial carcinoma cases 28(73%) were stained positive for beta hCG and only 9/37 (14%) were stained negative, of muscle invasive urothelial carcinoma cases 24(86%) were positive for beta hCG and only 4/28 (14%) showed negative staining (Table 3). Regarding expression of beta hCG in males, 12/55 (22%) showed negative staining and 43/55 (78%) were stained positive. In females only 1/10 (10%) were stained negative for beta hCG and 9/10 (90%) were positively stained.

Table 1: *Expression of β-hCG in cases of urothelial carcinoma*

ß-hCG Expression	Frequency	Percent
Negative	13	20.0
Positive	52	80.0
Total	**65**	**100.0**

Table 2: *Association of histologic grade and ß-hCG Expression in cases of urothelial carcinoma*

Histologic grade	ß-hCG Expression		
	Negative	Positive	Total
Low	8	27	35
High	5	25	30
Total	**13**	**52**	**65**

Chi-square. P-value = 0.534 (non-signficant).

Table 3: *Association of Muscle Invasion and ß-hCG Expression in cases of urothelial carcinoma*

Muscle invasion	ß-hCG Expression		
	Negative	Positive	Total
Non-Muscle invasive	9	28	37
Muscle Invasive	4	24	28
Total	**13**	**52**	**65**

Chi-square.
P-value = 0.316 (Non-significant)

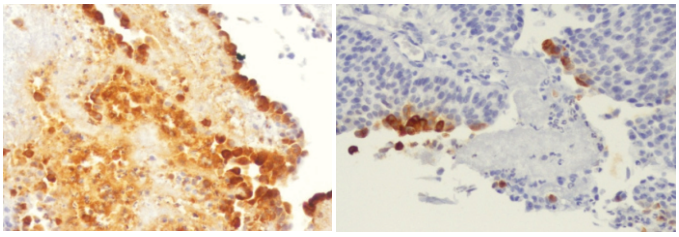

Figure 1: *Sections showing papillary urothelial neoplasm, high grade showing positive expression of beta hCG. (20x).*

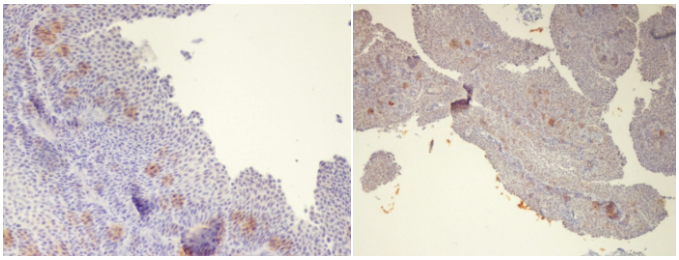

Figure 2: *Papillary urothelial neoplasm low grade showing positive expression of beta hCG (10x).*

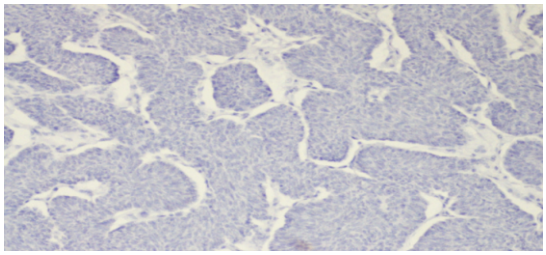

Figure 3: *Papillary urothelial neoplasm, High grade showing negative expression of beta hCG. (20X)*

Discussion

The present study was conducted to determine the expression of beta hCG in various grades of urothelial carcinoma. Urothelial carcinoma is primarily considered as a disease of old age. Age is a now a well-known, established and independent risk factor for urothelial carcinoma. Several studies conducted in various areas of world have highlighted the importance of age in development of urothelial carcinoma. In one series reported on bladder cancer in Iran showed that the mean age of patients was 61 years.[10] In another study conducted in India showed the mean age of presentation was 60 years.[11] The age at presentation of urothelial carcinoma observed in the biggest cancer center in Pakistan,

ShoukatKhanam Memorial Cancer and Research Centre, , is 55.5 years.[12] Study conducted in Center for Nuclear Medicine and Radiotherapy (CENAR) Quetta showed that the highest incidence of urothelial carcinoma is in 5th and 7th decade of life.[13] Several possible reasons have been identified to elaborate the relation between age and urothelial carcinoma. Smoking and occupations are closely linked to urothelial carcinoma, so as an age of individual increases, their contact to cumulative envorinmental exposure to carcinogens also increases. Several cellular events build up with age that can ultimately lead to neoplastic transformation. In elderly people the contact time of carcinogens, which are excreted in urine, increases because of incomplete emptying of bladder. Moreover, older people might drink less water because of exasperating voiding symptoms, that can also increases the concentration of the carcinogens in urine, and lastly because of decline in organ function in old age, there is reduced ability to detoxify the carcinogens ultimately increase the risk of development of urothelial carcinoma.[14] Our study showed the similar results regarding age where mean age of patients, presented with urothelial carcinoma were of 59.2 ± 11.6 years.

Majority of the patients suffered from urothelial carcinoma are male than females. In our study male to female ratio calculated was 5.6:1, which is similar to study conducted in central Punjab according to which male to female ratio was 3:1.[15] A study conducted in srilanka also showed male predominance with a male to female ratio of 5.7:1.[16] Envorinmental factors, Urination habits or hormonal imbalance are thought to be the major risk factors for increased incidence of urothelial carcinoma in males. As only a few women work in industrial areas so there is reduced exposure of females to carcinogens, can be considered as a possible reason for this sex disparity. Females avoid exposing the disease of genital tracts to someone due to social trends in our population, this might be one of the possible reasons for this reduced incidence of urothelial carcinoma in females. Female gender have a survival advantage in most of the cancers, however, this does not appear to be true in case of bladder cancer. Women constantly presents with more advanced stage of tumor and possess a worse oncologic outcome, despite of the higher occurrence of bladder cancer in males as discussed above, women consistently presents with more advanced staged tumors and worse oncologic outcomes at initial diagnosis. Female sex may now be considered as an independent risk as well as a prognostic factor for progression and mortality due to cancer at all stages (Non-Muscle invasive disease and muscle-invasive bladder cancer). In addition, various studies have suggested that there is a poorer response to Bacillus Calmette Guerin (BCG) in females.[17] According to one series reported that bladder cancer in female patients is diagnosed at higher age than in male. As the diagnosis of bladder cancer mainly depends upon symptoms so it can be a possible reason for delay in the diagnosis in women. Whereas painless hematuria or any other symptoms i.e dysuria, frequency in males may quickly gain attention and lead to diagnostic cystoscopy which is to done by a competent urologist, while the same symptoms in women may be confused with simple urinary tract infection, which further delays the essential diagnostic steps. Low quality of care for women may be an additional cause of gender inequalities.[18] In our study there were only 10 female patients, of which 6 were suffering from low grade urothelial carcinoma and only 4 have high grade disease. It is thought that estrogen have some protective effect in females at the initial stages of bladder cancer with superficial and less-aggressive tumors. But once the disease has progressed, the protective effects of estrogen seems to be lost, results in a overall decrease survival rate in female patients who have muscle-invasive disease and aggressive bladder cancer. Detrusor muscle invasion is the most important marker to calculate the prognosis of the urothelial carcinoma. Majority of the patients presents with superficial, non-invasive urothelial carcinoma. The data collected for our study showed the results regarding invasion. In this study, of total cases of urothelial carcinoma 57% were non muscle invasive and 43% were muscle invasive. The possible reason for this difference can be explained as muscular invasion is a complex phenomenon involving disruption and alteration of several molecular pathways.[19] Only few number of studies have been conducted on the expression of beta hCG in different grades of urothelial carcinoma. All have shown different results. According to Martin at al[20] the expression of beta hCG in urothelial carcinoma was 29% of all cases they studied. Shah et al[21] studied beta hCG expression in 104 cases of urothelial carcinoma and found positivity in only 12 (11.5%) cases. Venyo et al[22] studied 86 cases of urothellial carcinoma and found beta hCG immunostaining in 33(38.3%)

cases. Our studies showed positive beta hCG expression in 52 (80%) of all 65 cases of urothelial carcinoma stained. Dirnhofer and associates[23] found positive expression of beta hCG in 36% of all cases of urothelial carcinoma they studied and found that this harmone may act as a local growth factor in promoting cancer. Majority of the epithelial malignancies produce beta hCG but previously it is thought that its production is biologically insignificance. However, now it has been thought that beta hCG may undoubtedly affect the tumor growth and development. It has been suggested that β-hCG may acts in an autocrine and paracrine manner to up-regulate the proliferation and development of urinary bladder cancer cells. This may happen through manipulation of the structural affinity between β-hCG, transforming Growth Factor (TGF) and Platelet Derived Growth Factor (PDGF). In urothelial carcinoma cell lines, exposure to beta hCG may be a reason of development of resistance to TGF-β-induced apoptosis.[24] Beta hCG is also believed to promote angiogenesis through transforming growth factor pathway.[25] Beta hCG-hLH gene cluster on chromosome number 19 encodes the beta subunit of hCG. Iles and associates found out that beta-hCG expression by bladder tumours is as a consequence of modified gene regulation, neither a re-arrangement nor an amplification of this gene cluster.

Given the potential function of beta hCG in promoting the cancer cell lines through by shutting down the immune response, resisting apoptosis and promoting angiogenesis portends the poor outcome of the disease. Venyo et al[22] showed that beta hCG expression is positive in 16/60 (26.6%) low grade tumors and in 17/26 (65.3%) in high grade tumors concluding that expression of beta hCG increases as a tumor grade increases. Several studies have been done regarding expression of beta hCG in other epithelial tumors and have shown that hCG expression increases with tumor grade.

Bacchi and Co[14] found the same results, their study concluded that 39.1% of urothellial carcinoma patients with beta hCG expression showed tumor progression. Possible explaination of this higher proportion of β-hCG reactivity in patients showing muscle invasion could be on account of the theory of de-differentiation.

Conclusion

The results of this study indicates that Urothelial carcinoma is more prevalent among males. Majority of the patients presented with urothelial carcinoma were above 50 years of age. Patients with urothelial carcinoma expresses receptors for beta hCG in our population. The expression of beta hCG found to be almost similar in different grades of urothelial carcinoma. There is no significant difference of beta hCG expression between muscle invasive and non-muscle invasive carcinoma. Expression of beta hCG in both low and high grades was found to be almost equal so it may not be used as a prognostic marker.

Ethical Approval: The ethical review committee, Faisalabad Medical University approved the study vide letter No.48/ERC/FMU/2023-24/369.

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Authors' Contribution:

AA: Conception, data collection, and manuscript writing
AIK: Data collection and analysis
AQ: Analysis and Interpretation of data
MTBM: Critically review for important intellectual content
AN: Acquisition of data and manuscript revision
KMD: Data Collection

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