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Optimization of the Diagnosis of Prostate Cancer by Application of Immunohistochemistry in the Gezira State, Sudan

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Abstract:

Prostate cancer is the most common malignancy and the second cause of cancer related deaths in men. In Gezira Central Sudan, it is ranked first in the men's cancers list. The main objective of this study is to optimize the diagnosis of prostate cancer by Immunohistochemistry (IHC) using high molecular weight cytokeratin (antibody clone 34BE12) as a marker in all types of prostatic specimens, to compare the diagnosis, sensitivity and specificity,

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between H&E sections and IHC using the antibody clone 34BE12. Prostate tissue (samples from the bank) was diagnosed firstly using H&E stain, then the antibody clone 34BE12 was used & a second diagnosis using immunohistochemistry was done, finally the diagnoses were compared together. Laboratory analysis of selected 118 prostate samples showed the following: Statistically there was no significant difference between H&E stain and IHC using the antibody 34BE12 but the results of IHC were more accurate in the diagnosis of prostate cancer; 83 (70.3%) samples were diagnosed as benign using H&E stain, when diagnosed with IHC 81(68.6%) samples confirmed to be benign and 2(1.7%) samples previously diagnosed as benign with H&E stain, showed evidence of malignancy when using IHC (P-value=1.429<α=0.05). 34 (28.8%) samples were diagnosed as malignant and single sample (0.8%) diagnosed as a typical by using H&E stain, with IHC 37(31.4%) samples were confirmed to be malignant including the two(1.7%) samples which were previously diagnosed as benign with H&E stain and the single sample(0.8%) which was previously diagnosed as atypical with H&E stain (P-value=1.414<α=0.05). This means that atypia is decreased by the antibody 34BE12 from 0.8% to zero. The sensitivity of H&E was 94.8% and the specificity was 96.4%, the sensitivity of IHC was 100% and the specificity was 100%. In conclusion IHC using the antibody 34BE12 is superior to H&E stain for diagnosis of prostate cancer particularly in small biopsies & atypical cases. Further studies should be done in the diagnosis prostate cancer using more than one antibody.

Key words: Prostate cancer diagnosis; H&E; IHC; Sudan.

1. INTRODUCTION

Prostate cancer is the most common extracutaneous malignancy and the second cause of cancer related deaths in men. In Gezira Central Sudan, it is number one cancer in men. In some cases it is not easy to diagnose prostate cancer with enough confidence; therefore the application of Immunohistochemistry to distinguish prostate cancer from

benign mimickers is very important. The aims of this study to facilitate the optimal diagnosis of prostate cancer by Immunohistochemistry using high molecular weight cytokeratin (HMWCK) antibody clone 34BE12 as a marker, to compare the diagnosis between H/E sections and the antibody clone 34βE12, to compare the sensitivity and specificity of the two methods, and to compare the cost and duration of the two methods Vs accuracy of diagnosis.

Prostatic Intraepithelial Neoplasia **(**PIN) is divided in to two grades; low grade and high grade. The transformation from low grade to high grade and invasive carcinoma is characterized by basal cell layer disruption, progressive loss of markers of secretory differentiation, and increasing nuclear and nucleolar abnormalities, proliferative activity, micro vessel density, genetic instability and DNA content [(1)]. Therefore it is postulated that PIN is derived from transformed stem cell populations located in the basal cell layer [(2)]. Low grade PIN is characterized by the presence of variable nuclear enlargement and irregular cell spacing resulting in nuclear stratification and crowding. High grade is considered the precursor of most prostate carcinoma. In high grade PIN the proliferating epithelial cells have cytologic changes mimicking carcinoma, including nuclear and nucleolar enlargement, the presence of prominent nucleoli, often multiple, is helpful in the diagnosis [(3)] [(4)]. At the periphery of PIN, the basal cell layer, which is discontinuous this is distinctive feature that half of acini with high grade PIN, but requires immunohistochemical stain (high molecular weight cytokeratin) for its identification [(5)]. PIN shares proliferation and differentiation disorders with other well established epithelial lesions like a typical adenomatous hyperplasia and sclerosing adenosis.

The atypical adenomatous hyperplasia retains the polarized pattern of its proliferative compartment and expresses bcl-2 exclusively in basal cells, it lacks typical

premalignant proliferative disorders of PIN [6] [7]. Sclerosing adenosis occurs in the transition zone and shares certain features with well differentiated adenocarcinoma, but it retains the basal cell layer lining [8]. The Ab clone 34ßE12 directed to CK1, CK5, CK10, CK14, and unknown CK subtypes, is useful for detection of HMWCKs. Due to their reactivity to unknown CK subtypes, 34ßE12 also reacts with some simple epithelial cells of breast, colon, biliary tract, kidney but not prostate[9].H&E stain is the most widely used stain in medical diagnosis. The staining method involves application of the basic dye Haematoxylin, which colors basophilic structures with blue purple hue, and alcohol- based acidic eosinY, which colors eosinophilic structures bright pink [10].

2. DEFINITION OF STUDY AND STUDY AREA

This was a prospective and comparison study between the ordinary histological method using H&E stain and immunohistochemical method using HMWCK antibody clone 34βE12 in the diagnosis of prostate cancer. The study was conducted during the period from 2009 to 2012.

Study samples were selected from Gezira Medical Laboratory. University of Gezira, located in Gezira state, Central Sudan. These samples were brought from all over the catchment area for different stains.

3. THE ORDINARY HAEMATOXYLIN AND EOSIN (H&E) STAIN

The formalin fixed specimens of prostate samples were dewaxed, hydrated in descending grades of alcohol concentration, at 100%, 95% through 70% to distilled water for 2 minutes in each stage. For staining of the nucleus, the sections treated with Mayer's Haematoxylin for 8 minutes and

differentiated by rinsing in acid alcohol for seconds, bluing in running tap water for 8minutes, counterstaining in Eosin for 1 minute, and rinsed in water. The sections dehydrated in 70% alcohol through 95% and 100% alcohol, and then blotted in a filter paper, cleared in xylene and mounted in DPX, after that the smears were ready for microscopic examination.

Interpretation of the results: Nucleus; deep blue colour. Cytoplasm and background tissue; pink colour. RBCs; orange colour [(11)].

4. THE IMMUNOHISTOCHEMISTRY METHOD (STREPT AVIDIN- BIOTIN)

Paraffin sections were put in albumenized glass slides and then the slides placed in oven at 56c° for 30 minutes. The sections were deparaffinized in two changes of xylene for 4 min in each. The sections were rehydrated in decreasing grades of ethanol for 4 min in each, 100%, 90%, and 70% ethanol and then to distill water. Then tissue in the sides was circled with a diamond marker. The retrieval was done by proteinase K enzyme for 5 minutes. The slides rinsed in distill water and washed in tris buffer saline (TBS) for 5 minutes. The slides were placed in 3% solution of hydrogen peroxide (blocking solution) for 5 min. The slides were washed in TBS for 5 min.

The slides were placed in humidity chamber. The circles were covered with primary antibody (Ab) for 20 minutes at room temperature. The slides washed in two changes of TBS for 5 minutes in each.

The biotinylated secondary Ab, or label, or polymer was added for 20 minutes at room temperature. The slides were washed in three changes of TBS for 5 min in each. The streptavidin enzyme complex was added for 25 minutes. The slides were washed with TBS for 5 minutes. Drops of chromogenic solution (DAB) (20 microlitre of chromogen +1000

microlitre substrate) were added for 10 minutes. The slides washed in TBS for 5 minutes. Counter-staining with haematoxylin stain for 2 minutes. Washing of the slides with running tap water (blueing) for 2 minutes. The slides dehydrated with increasing grades of ethanol, twice for 2 min in each of 70%, 90%, and absolute ethanol. let to dry, or blotted by filter paper. Clearing of slides with xylene and cover slipping of sections with mounting medium.

Interpretation of the results: Reaction location; as brown colour in the cytoplasm. The basal cells should show strong cytoplasmic staining reaction, with no reaction of the secretory cells [(12)].

5. RESULTS

5.1 The histological diagnosis of H&E stain and IHC Using the Antibody 34*BE*12

Out of 118 cases, 83 (70.3%) samples were diagnosed as benign using H&E stain. With IHC 81 (68.6%) cases confirmed to be benign and 2 (1.7%) cases previously diagnosed as benign with H&E stain, showed evidence of malignancy when using IHC. (P-value=1.429 <α=0.05).

Thirty four (28.8%) cases were diagnosed as malignant using H&E stain. With IHC 37 (31.4%) cases were confirmed to be malignant including the 2 (1.7%) cases which were previously diagnosed as benign with H&E stain and the single case (0.8%) which was previously diagnosed as atypical with H&E stain, was confirmed to be malignant by using IHC. (P-value=1.414 <α=0.05) (table1).

5.2 The Sensitivity, Specificity, Positive and Negative Predictive Value of H&E and IHC Using the Antibody 34*BE*12

H&E was 94.8% sensitive, 96.4% specific, 92.5% Positive Predictive Value (PPV), and 97.5% Negative Predictive Value (NPP). IHC was 100% sensitive, 100% specific, 100% PPV, and 100% NPP (table 2).

5.3 The Duration and Reagent Cost of H&E Stain and IHC using the Antibody 34*BE*12

H&E stain (36 hr) was rapid than IHC (42 hrs) in performing the staining method. H&E stain (20 SDG/sample) was cheaper than IHC method (80 SDG/sample) (table 3).

Table 1. The histological diagnosis of H&E stain and IHC Using the Antibody 34*BE*12

Stain	Benign	Malignant	Atypical	Total	Chi-square (χ^2)	Degree of Freedom
H&E	83(70.3%)	34(28.8%)	1(0.8%)	118(100%)	86.559	2
IHC	81(68.6%)	37(31.4%)		118(100%)	16.401	1

Table 2. The Sensitivity, Specificity, PPV, and NPP of H&E and IHC using the antibody 34*BE*12

Stain	Sensitivity	Specificity	PPV	NPV
H&E	94.8%	96.4%	92.5%	97.5%
IHC	100%	100%	100%	100%

Table3. The duration and reagent cost of H&E stain and IHC using the antibody 34*BE*12

Method	Duration/ hrs	Cost (SDG/Slide)
H&E	36	80
IHC	42	20

6. DISCUSSION

Statistically there was no significant difference between H&E and IHC using Ab34*BE*12 (p-value=1.429 <α=0.05 for benign

cases, and p-value=1.414 <α=0.05 for malignant cases). But the results of IHC were more accurate. The diagnosis of three cases (2.5%) had been changed when using IHC, in which (2) cases were previously diagnosed as benign with H&E stain, showed evidence of malignancy with IHC, and (one) case which was previously diagnosed as atypical with H&E stain, was confirmed to be malignant by using IHC. The a typia in H&E was 0.8% (one sample), when using Ab34*BE*12 the atypia was decreased from 0.8% to zero.

These differences in the results between H&E and IHC because there is no single morphological feature specific for prostate cancer and many conditions can mimic prostate cancer and need differentiation, like atypical adenomatous hyperplasia of the prostate, which occurs in the transition zone and some few cases of well differentiated small adenocarcinoma. Also sclerosing adenosis occurs in the transition zone and shares certain features with well differentiated adenocarcinoma, but it retains the basal cell layer lining.

H&E is the routinely used stain in histopathology. The stain depends on acid –base reaction between the stain and tissues. The staining method involves application of the basic dye Haematoxylin, which colours basophilic structures with blue purple hue, and alcohol- based acidic eosinY, which colours eosinophilic structures bright pink. Immunohistochemistry is a technique for localizing and visualizing an antigen in a tissue section by using an antibody specific for the target antigen. The Ab34*BE*12 specially stains the basal cells of the prostate. So it was more specific than H&E. This was well reflected in our results.

These results agree with many authors like Lin YC, Ro JY, and Logothetis CGb, they published that the differential diagnosis of well differentiated carcinoma includes atypical adenomatous hyperplasia and sclerosing adenosis which occur

in the transition zone and share certain features with well differentiated adenocarcinoma. The monoclonal antibody 34ßE12 and K-903 is helpful in demonstrating the basal cell layer in sclerosing adenosis [8].

Rosai J, concluded that immunohistochemistry plays an important role in diagnosis of prostate cancer. It helps to differentiate malignant glands from benign lesions, especially for morphologically equivocal glandular alterations in small core biopsy specimens. The immunohistochemical diagnosis of prostate cancer largely depends on panels of markers because no absolutely specific and sensitive marker for prostate cancer has yet been discovered [13].

Varma M, Jasani B, concluded that immunohistochemistry with the antibody 34BE12 (HMWCK) is the most useful in the diagnosis of prostate cancer. This monoclonal antibody specially stains the basal cells of prostate in formalin fixed tissues [14].

Varma M, *et al*, reported that there is no well-established positive immuno marker for urothelial carcinoma. They evaluated the utility of high molecular weight cytokeratin (HMWCK) antibody clone 34βE12 in differentiating high grade invasive urothelial carcinoma from prostate cancer. And found that HMWCK antibody clone 34BE12, when used with microwave heat retrieval, is a very sensitive positive marker for high grade invasive urothelial carcinoma [15].

Masaru Shipn, *et al*, evaluated the utility of immunohistochemical detection of high molecular weight cytokeratin for differential diagnosis of proliferative conditions of prostate. Differential diagnosis of adenocarcinoma from other proliferative conditions in prostate is often problematic. Immunohistochemistry using HMWCK antibody clone 34BE12, especially present in basal cells of the prostate, can demonstrate the presence or absence of these cells in

proliferating glands, therefore the use of this antibody is indispensable in the diagnosis of equivocal prostate lesions [16].

Peng Yahui MS, *et al*, reported an automated computer technique for detection of prostate cancer in prostate tissue sections using cocktail of antibodies included; alpha-methylacyl-CoA racemase, p63, and HMWCK. They found that if high grade prostatic intraepithelial neoplasia and atypical cases were included, the sensitivity and specificity were 85% for alpha-methylacyl-CoA racemase, and 89% for both, p63 and HMWCK [17].

Ab34*BE*12 is very important in the diagnosis of a typical glands (the a typia in H&E was 0.8%, when using Ab34*BE*12 the atypia was decreased from 0.8% to zero), few malignant glands, perineural invasion, crushed and inflamed. These cases cause difficulty in microscopic examination with H&E but they are very clear with IHC.

These results agree with many authors like Bostwick DG, reported that there are problems sometimes in interpreting the significance of few negatively stained glands, and also other problems include the interpretation of positive glands, in some cases it difficult to determine whether they represent entrapped benign glands. Therefore the use of antibody 34ßE12 (HMWCK) is very important because it reduces atypical diagnosis from 8% to <1% in prostate core needle biopsies [4].

Hillel Kahance MD, *et al*, evaluated the use of HMWCK in staining of prostate tissues, and found that HMWCK selectively labels the basal cells, and its use decreased the rate of atypical prostate biopsies from 8.3% to 0.4% at a negligible cost to the pathologist and patient [18].

H&E and IHC have many steps (tissues fixation, processing, microtoming and staining), which take time to be performed. IHC method has a lot of steps, which need more time than H&E stain. H&E stain is commercially available in Sudan, but IHC reagent is not available commercially due to its

high reagent cost and in Sudan it is ordered from abroad on demand and till now not used (this is the first time to be used in Sudan).

7. CONCLUSION

IHC using the antibody 34*BE*12 is superior to H/E for diagnosis of prostate cancer particularly in small biopsies & atypical cases. Also the antibody 34*BE*12 is more sensitive and specific than H/E stain, but it is more costly and time consuming than H/E stain.

Due to its high accuracy, IHC using the antibody 34*BE*12 is recommended to be used with H&E stain to confirm the diagnosis of prostate cancer. Further studies should be done in prostate diseases using more than one antibody.

ETHICAL APPROVAL

Tissue blocks were used (the samples from the Bank), numbered samples, no patients' name.

Ethical approval for this study was obtained from the research ethical committee from the Gezira State Ministry of Health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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