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International Journal of Current Research
Vol. 8, Issue, 12, pp.43809-43812, December, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

DIAGNOSTIC UTILITY OF ANTIBODY COCKTAIL EXPRESSION IN DIFFERENTIATING PROSTATIC INTRAEPITHELIAL NEOPLASIA WITH CARCINOMA

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ARTICLE INFO

Article History:
Received 23rd September, 2016
Received in revised form
27th October, 2016
Accepted 20th November, 2016
Published online 30th December, 2016

Key words:

Prostate, Hyperplasia,
Intraepithelial neoplasia,
Adenocarcinoma,
Immunohistochemistry,
Antibody cocktail.

ABSTRACT

Objectives Histopathologists often face difficulties in diagnosing Prostatic Intraepithelial Neoplasia (PIN) or borderline Adenocarcinoma (PAC) cases on the basis of hematoxylin and eosin (H & E) stain alone. Recent immunohistochemistry (IHC) studies have shown ERG overexpression linkage with carcinomatous changes whereas CK5 expression in normal basal cell layers in prostate is an established fact. Limited review of literature reveals high specificity of multiplex antibody cocktail mixtures comprising of neoplastic acinar as well as basal cell markers for demarcating prostatic lesions.

Aims: Objective of this study is to evaluate diagnostic efficacy of multiplex antibody cocktail mixture of ERG and CK5 in delineating PIN and PAC cases.

Settings and Design: A sample size of 30 cases was targeted including Hyperplasia (BPH), PIN and PAC. All the relevant clinical details including serum PSA were recorded.

Methods and Materials: Cases were subjected to routine H & E staining along with IHC evaluation by multiplex antibody cocktail mixture of ERG & CK5.

Results: Out of 30 cases, 11 were labeled as BPH, 9 PIN and rest 10 as PAC on H & E staining. Of 11 BPH, 10 showed CK5 positivity alone, while 1 showed both ERG & CK5 coexpression. Subsequently diagnosis was revised as PIN. Of 9 PIN, 5 were ERG negative and CK5 positive; hence diagnosis revised as BPH. 2 were ERG positive and CK5 negative; hence rechristened as PAC. Rests 2 were consistent with PIN in form of ERG negativity and CK5 positivity. Of 10 PAC, all were CK5 negative, but only 7 were ERG positive. On review, rest 3 ERG negative cases had high Gleason's score.

Conclusion: Immunohistochemical evaluation by multiplex antibody cocktail mixture of ERG and CK5 has great utility in resolving diagnostic and prognostic problems of PIN and PAC cases. It has potential to be considered as screening immunohistochemical module for reporting of prostatic biopsies.

Citation: Piyush Sagar, Atin Singhai, Suresh Babu, Nuzhat Husain and Sankhwar, S.N. 2016. "Diagnostic utility of antibody cocktail expression in differentiating prostatic intraepithelial neoplasia with carcinoma", *International Journal of Current Research*, 8, (12), 43809-43812.

INTRODUCTION

Histopathologists, particularly those in their initial years of practice or with lesser experience often face difficulties in diagnosing and differentiating prostatic intraepithelial neoplasia (PIN) with low grade/borderline prostatic adenocarcinoma (PAC) cases. PIN is a condition characterized by neoplastic growth of columnar epithelial cells within pre-existing benign prostatic acini or ducts, with intact basal cell layer. PAC requires demonstration of stromal invasion for diagnosis. Borderline / low grade PAC cases may show only focal or no evidence at all for stromal invasion in small prostatic biopsy samples received for reporting (Ware, 1994 and Nelson, 2003). It has also been postulated that if there is increased frequency / extent / severity of PIN like changes, without any demonstrable stromal invasion, such cases should be treated as PAC (Ware, 1994 and Berry, 1984). However in practice, the above mentioned scenarios pose a really diagnostic challenge for the reporting histopathologists and thereby create a dilemma for planning of management of patient too (Kearse, 1993 and Brawer, 1992). During last few decades, quite a few immunohistochemical markers have come up but their utility has been limited to addressal of benign verses frank PAC cases only. PIN cases suspicious for non sampled PAC still had no addressal (Voltaggio, 2016 and Merrimen, 2013). ERG (ETS related gene) discovered in 2005, is an oncogene, a member of ETS gene family that fuses with

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androgen hormone regulated gene TMPRSS2 and causes uncontrolled growth of tumor cells. It has also been demonstrated that ERG oncoprotein monoclonal antibody has high degree of specificity in PAC, with no sign of expression in non PAC cases (Furusato, 2010; Park, 2010 and Petrovics, 2005). CK5 is a high molecular weight cytokeratin and a well-established immunohistochemical marker expressed by normal basal cell layer of prostatic glands. Its pan expression across intact basal cell layer lining of prostate clearly rules out possibility of PAC (Trpkov *et al*., 2009 and Hameed, 2005). Since last few years use of immunohistochemistry cocktail panels have increasingly been in use which allows simultaneous detection of two or more different antigens within one tissue section by sequential staining (Furusato, 2010 and Trpkov, 2009).[1] In present study we employed use of multiplex immunohistochemistry cocktail mixture of ERG & CK5 across all the received prostatic biopsy samples to study its expression behaviour in different possibilities like benign prostatic hyperplasia (BPH), PIN or PAC cases in order to establish whether this can be utilized as a base line IHC panel in suspected borderline PAC cases.

MATERIALS AND METHODS

Samples of 30 cases, both TURP and TRUS guided were registered which included clinically suspicious BPH, PIN & PAC cases. All the relevant clinical details along with serum PSA were recorded, however were not revealed to reporting histopathologists to prevent analytical bias. All samples were subjected to routine haematoxylin and eosin (H & E) staining. The stained biopsies were studied by three pathologists (AS, SB, MK) independently. Diagnosis on H & E light microscopy was framed with consensus by at least two pathologists. All the above cases were further subjected to multiplex immunohistochemistry cocktail mixture of ERG & CK5. The antibody cocktail comprising of ERG and CK5 was procured from Biocare Medical (catalogue number API 437 DSAA). This sequential double stain involved use of ERG as primary and CK5 as secondary antibody. Primary antibody was subjected to horseradish peroxidase (HRP) detection system followed by denaturation to eliminate cross reactivity from application of second detection system. Second primary antibody is then introduced, followed by application of alkaline phosphatase (AP) detection system. Visualization of antigens was achieved with DAB & red chromogens. DAB chromogen was picked up by ERG positive cells while red chromogen was revealed by CK5 positive cells. Cocktail expression, as per review of literature was expected as: ERG negative CK5 positive (BPH), ERG positive CK5 positive (PIN), and ERG positive CK5 negative (PAC). Statistical analysis was done by Graph Pad Prism version 5.

RESULTS

Serum PSA levels of the received cases were arbitrarily divided in to 4 subgroups as: I (0-4 ng/ml), II (4-8 ng/ml), III (8-12 ng/ml), IV (>12 ng/ml). The distribution of BPH cases was as: 27% (I), 64% (II), Nil (III) & 9%(IV). PIN cases had their serum PSA levels as: 11% (I & IV each), 44% (II) and 34% (III). 80% of PAC cases were in group IV while 10% cases each were in group I & II. (Table 1) However serum PSA levels were not disclosed till complete immunohistochemical workup. In diagnosis by H & E reporting alone, there was uniform consensus by all three pathologists in 28 cases while in 2, there was one disagreement each. On this basis, the consortium of 30 cases consisted of 11 BPH, 09 PIN and 10 PAC cases. Histopathological diagnosis on basis of H & E staining correlated well with (ERG + CK5) cocktail expression in BPH (10 of 11) and PAC (7 of 10) cases but for PIN, there was poor concordance (2 of 9). It is also to be noted that 3 of PAC cases showed no reactivity for either ERG or CK5. (Figure 1, Table 2).

Table 1. PSA scores in different groups

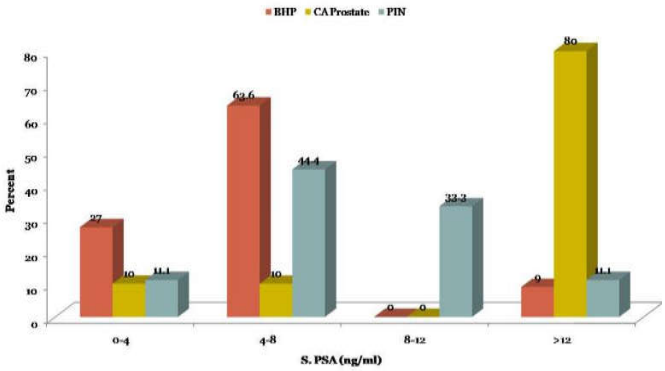

Table 2. IHC pattern in different groups

ENTITY	Number of Cases	ERG Negative CK5 Positive	ERG Positive CK5 Positive	ERG Positive CK5 Negative	ERG Negative CK5 Negative
BPH	11	10	01	Nil	Nil
PIN	09	05	02	02	Nil
PAC	10	Nil	Nil	07	03
p value : 0.005			Chi^2 value : 10.344		

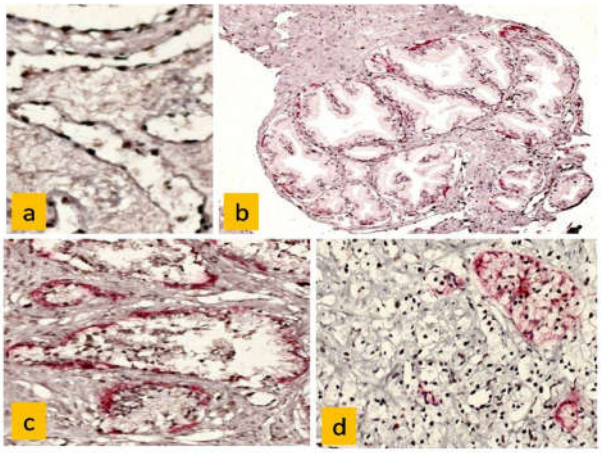

Figure 1. (a) Positive ERG across vessels : Internal Control (b) BPH : ERG Negative CK5 positive (c) PIN : ERG Positive CK5 Positive (d) PAC : ERG Positive CK5 negative

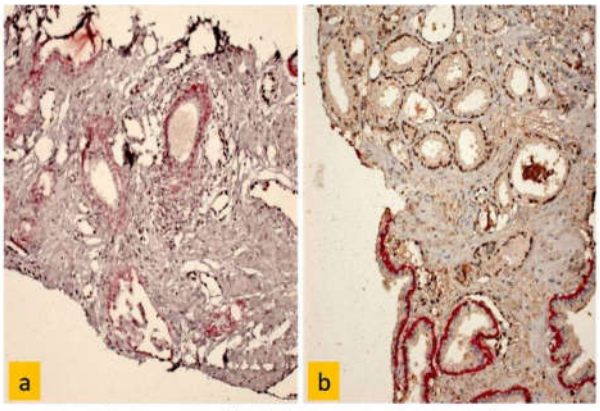

Figure 2: Fallacious cases (a) Labeled as PIN on histopathology, confirmed as BPH by immunohistochemistry (b) Labeled as PIN on histopathology, confirmed as PAC by immunohistochemistry

All the fallacious cases were reassessed this time involving a fourth histopathologist (NH) too. One initially diagnosed BPH cases turned out to be positive for both ERG & CK5 expression. Its total serum PSA level was found to be 11.4 ng/ml. Further subfraction revealed free PSA level of about 60% of total PSA value. Deep cut of biopsy block was also studied and it did reveal hyperplastic columnar epithelium with intact basal cell layer. Therefore, this case was relabelled as PIN. Of the 9 histologically diagnosed PIN cases 7 of them did not correlate with the expected immunohistochemical expression. 5 cases showed immunohistochemistry consistent with BPH. On retrospective analysis of their serum PSA levels, one had its value < 4 ng/ml while 4 of them ranged between 4-8 ng/ml. Their free PSA values too were well above 25% in all the cases. Histologically too, there was evidence of mild tufting at fair member of places, but no apparent nuclear pleomorphism or atypia. Subsequently after pan consensus all the five cases were rechristened as BPH with sporadic low grade PIN like changes and advised regular clinical follow up along with Serum PSA estimation. 2 of the diagnosed PIN cases showed immunohistochemistry profile in concordance with PAC. Both the cases had borderline serum PSA values, 10.1 and 9.4 ng/ml respectively. Free PSA value of only one case with 10.1 ng/ml serum PSA was available and it was 8 % of the total value. On careful retrospective microscopic examination even there was no evidence of stromal invasion. But keeping in view immunohistochemistry findings and serum PSA levels with probability of unsampled representative foci in biopsies, the diagnosis was relabelled as low grade PAC with Gleason's score 2+2 each in both the cases (Figure 2).

DISCUSSION

Lesions of prostatic gland constitute a significant proportion of morbidity & mortality in male population across the world. If age specific incidence are to be considered the data surge is even more in the elderly population (Berry, 1984 and Kearse, 1993). BPH and PAC with high Gleason's scores are easy to diagnose and face a very low inter observer variability. However PIN & PAC with low Gleason's score are not always easy to diagnose exactly and there exist a significant inter observer variability attributed by overlap of the histopatholgical findings and /or probability of unsampled diagnostic pathological sites in the received scanty TRUS guided specimens. Most of the immunohistochemical diagnostic modalities in practice characterize either BPH or PAC. As PIN is considered a precursor of PAC, it is expected to express markers for both BPH & PAC (Bostwick, 1978 and Brawer, 1992). With this novel concept multiplex cocktail mixture comprising of primary antibodies to ERS & CK5 was employed to study their expression in PIN and to differentiate them with PAC, especially ones with low grade Gleason's scores. Results obtained were quite in consistence with expected logistics.

On routine histopathology BPH & PAC cases had no inter observer variability while PIN cases showed difference in opinion in 2 cases. Results of ERG and CKS cocktail expression were fairly consistent with histologically diagnosed BPH & PAC cases, however only 2 of the 9 PIN cases showed concordant inmmuhistochemical expression. This led to review and reassessment of the non-consistent cases and final diagnosis had to be altered in sync with their immunohistochemical expression. The results achieved statistical significance too (Chi^2 value: 10.344, p value: 0.005) confirming that use of multiplex (ERG + CK5) antibody cocktail offers a better and realistic screening module for prostatic biopsies. Immunostaining by multiplex antibody cocktail mixture offers clear advantages over the conventional single antibody use. Particularly in the present study use of cocktail mixture proved to be quite efficacious to enable easy detection of atypical acini and basal cells by use of different coloured chromogens on common pathological representative areas.10,15 Besides being an easy and simple manoeuvre, it also prevented potential loss of representation by evaluating a single slide (Voltaggio, 2016 and Trpkov, 2009).

This proved to be quite beneficiary especially in differentiating PIN with low grade PAC cases. ERG as quoted by review of literature lacks high sensitivity it but has high specifity. Studies have reported its sensitivity ranging from 15 - 72 %, when used as a single marker (Petrovics, 2005 and Kumar-Sinha, 2008). However combined staining of ERG with a basal cell marker tends to enhance its diagnostic value (Trpkov *et al*., 2009 and Adamo, 2016). More over such cocktails have been reported to be more sensitive in picking up high grade PIN cases, when compared with other prostatic immunohistochemistry markers (Voltaggio, 2016 and Furusato, 2010). In present study immunostaining by (ERG + CK5) antibody cocktail mixture performed a crucial and deciding role in demarcation of PIN with low grade PAC cases. Accordingly this further helped in delineating specific management plans. Use of antibody cocktail mixture certainly offers an easy, precise and less time consuming approach in defining lesions with suspicious / borderline neoplastic histomorphology, where significant interobserver perception variability exists.

Conclusion

To conclude, use of an antibody cocktail mixture comprising of basal as well as neoplastic acinar cell markers may be considered as a screening immunohistochemical approach for reporting of prostatic biopsies as it aids in precise diagnosis of fallacious cases and thereby saves clinician from dilemma of uncertainty in planning management of such cases.

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