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Original Research Article

Diagnostic Utility of Specific Red Cell Adherence (SRCA) Test in Various Prostatic Lesions

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ABSTRACT

Introduction: In malignant transformation of tumors, the A and B blood group antigens which are normally present on various body tissues are lost. We have made an attempt to differentiate prostatic lesions between benign, premalignant and malignant lesions through this study. As histological assessment is not an accurate indicator of risk of progression of malignancy. The loss of ABH reactivity correlated significantly with the stage of tumor development and histological grading of malignancy. We have studied is antigens A, B, and H in benign, premalignant and malignant lesions in prostatic lesions by use of Specific Red Cell Adherence (SRCA) test.
Materials and methods: A total of 41 prostatic lesions were collected between Jan2012- June 2012 and Specific Red Cell Adherence (SRCA) test was performed on the lesions. The results were then evaluated.
Results: A total of 41 prostatic cases – 34 cases (82.92%) benign lesions showed strongly positive at the epithelium of hyperplastic acini, 5cases (12.19%) premalignant showed weakly positive and 2 cases (4.87%) malignant lesions showed uniformly negative. We found statistically significant relationship of antigenic expression in benign, premalignant and malignant lesions.
Conclusion: Specific Red Cell Adherence (SRCA) test is an early indicator of risk of progression of malignancy. It also helps in predicting recurrences and overall survival rate of patients. SRCA technique offers advantage of being highly economical, cost-effective, and reliable as compared to immuno peroxidase, flow cytometry and immunohistochemical staining. It not only improves accuracy but also reproducibility as it does not require extensive training.
Keywords: Specific red cell Adherence (SRCA), Isoantigens, Prostatic lesions.

INTRODUCTION

The blood group antigens [A, B and O (H)] are expressed in normal tissues and body cavity fluids of the human body other than red blood cells known as isoantigens as per Manju Sharma et al [(1)] and demonstration of these isoantigens was done through the procedure of Mixed cell Agglutination reaction (MCAR) technique in cancer by Kovarik S et al. [(2)] Examples of these include endothelial cells lining blood and lymph vessels, squamous epithelium of

tongue and cervix, columnar epithelium of gastric and bronchial mucosa and transitional lining cells of urinary bladder. [2, 3, 4]

The diagnosis of prostatic lesions especially the prostatic adenocarcinoma is challenging and surprising to the pathologist. As these carcinomas have various histomorphlogical patterns of presentation with minimal cytological and architectural atypia in limited tissue fragment like needle biopsy. The histomorphological variants in prostatic tumor like Atrophic, foamy gland, pseudohyperplastic and certain subtypes of ductal adenocarcinoma represent the most common causes of under-diagnosed cancer when the pathologist is not familiar with these entities, and therefore those are called "pseudobenign" carcinomas [1] or carcinomas mimicking benign lesions. [6-8]

Hence Specific Red Cell Adherence test can be used as alternative to other diagnostic techniques as it is economically cheap, cost-effective, and easily reproducible. Specific Red Cell Adherence test can be used as an adjuvant test and supplemented routine morphological study in diagnosis of pseudo benign carcinomas.

Aim and Objectives

- To study isoantigens A, B and H in benign, premalignant and malignant lesions of prostate by use of Specific Red Cell Adherence (SRCA) test.
- Diagnostic utility of Specific Red Cell Adherence (SRCA) test in various Prostatic Lesions.

MATERIALS AND METHODS

A total of 41 prostatic lesions were evaluated as benign premalignant and malignant by histopathological examination during the period of 6 months from Jan 2012 to June 2012.

Tissue preparation:

The biopsies included in this study were TURP and prostatectomy specimens. All biopsies specimens were fixed in 10 % buffered formaldehyde, paraffin embedded, sectioned at 4 microns, and stained with hematoxylin and eosin. Sections (4 microns) from the tumor biopsies were placed on albumin-coated slides. Sections were deparaffinized in xylene and brought to water through graded ethanol (100%).

Specific red cell adherence test:

Specific red cell adherence test was performed on paraffin embedded sections to detect the intensity of isoantigens A, B and H (O) on the epithelial cell surface by a three layer sandwich technique, as described by Walkers J [9] and Strauchen JA et al. [10] Commercially available Anti A, Anti B, and Anti AB antisera from Span Diagnostic Limited and *Ulex europaeus* lectin (Anti H) were used in our study. These seras had average agglutination titres of 512. Slides of 4-5 micron section were deparaffinized and brought to water.

1. The slides were immersed in Tris buffered saline 0.05 M (pH 7.4) for 30 minutes.
2. The slides were covered with isologous antisera with Anti-A, -B and -O according to patient's blood group, and incubated for one hour with in a moist chamber at room temperature.
3. The slides were then dipped in Tris buffered saline three times with occasional stirring to remove the unreacted antisera.
4. A few drops of 2-5% isologous indicator RBC's suspension were added to the sections and incubated for 20 minutes in group A or B and one hour for group O.
5. The slides were inverted over a support in a Petridis containing Tris buffered saline such that the undersurface of the slide just touched the solution, and kept for five minutes to settle down the unreacted RBCs.
6. The slides were observed under low power magnification and photographed immediately.

7. The test had its own positive internal control like RBCs, endothelial cells of blood vessels and negative internal control was connective tissue like fat and fibrous tissue.

Interpretation:

In the present study, isoantigenicity of the epithelium was said to be completely preserved when there was continuous chain of adherence of RBCs was seen at the epithelium in benign prostatic lesions (Figure1).

Partially preserved isoantigenicity of the epithelium was said when there was focal adherence of RBCs at the epithelium in premalignant prostatic lesions (Figure 2, 3).

Total deletion of isoantigenicity of the epithelium was said when there was total absence of adherence of RBCs at the epithelium in malignant prostatic lesions (Figure 4).

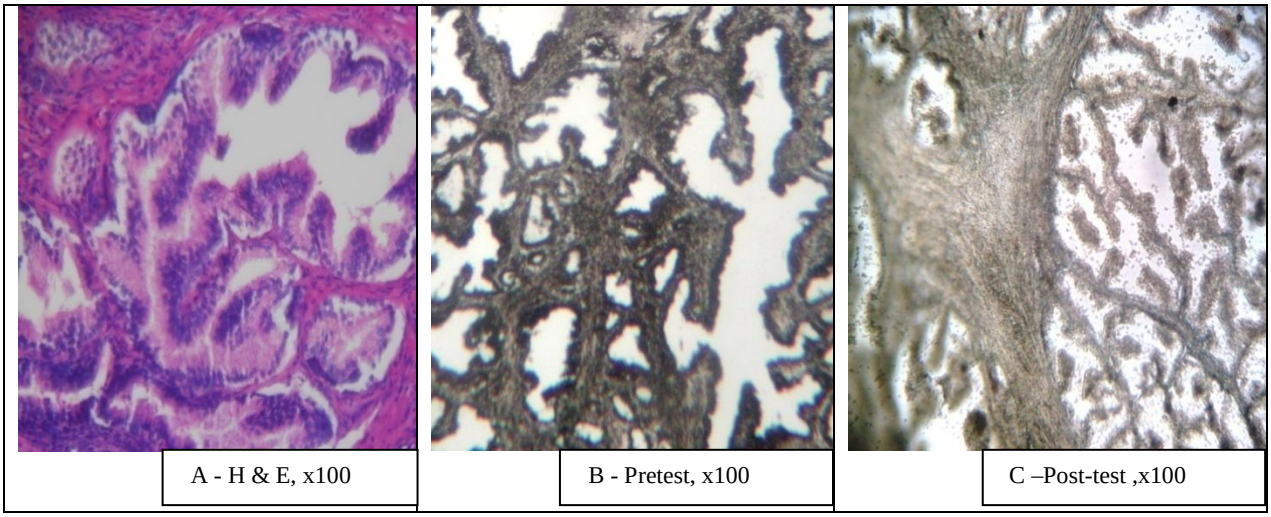

Figure 1.Benign Lesion – Benign prostatic hyperplasia.
A - H & E Stain(x100)
B - Unstained deparaffinised section (before test x100).
C – Post SRCA test shows (x100) – Strong adhesion of RBCs at the epithelium.

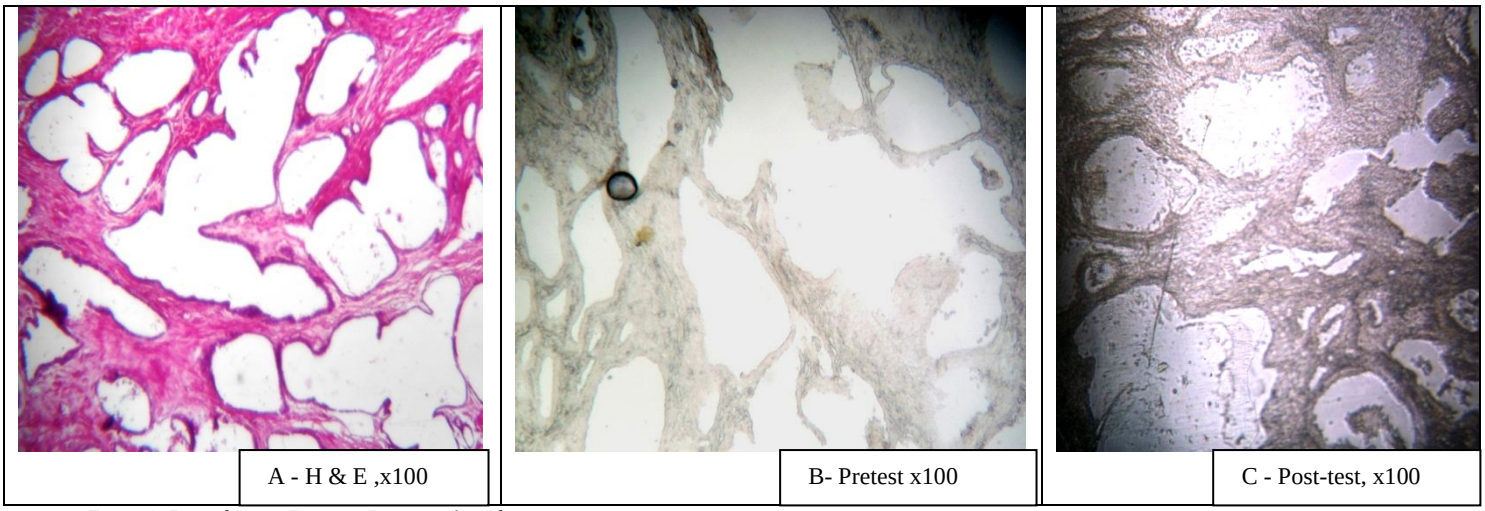

Figure 2. Premalignant Lesion – Prostatic Atrophy.
A - H & E Stain(x100)
B - Unstained deparaffinised section (before test x100).
C – Post SRCA test shows (x100) - focal/partial adhesion of RBCs at the epithelium.

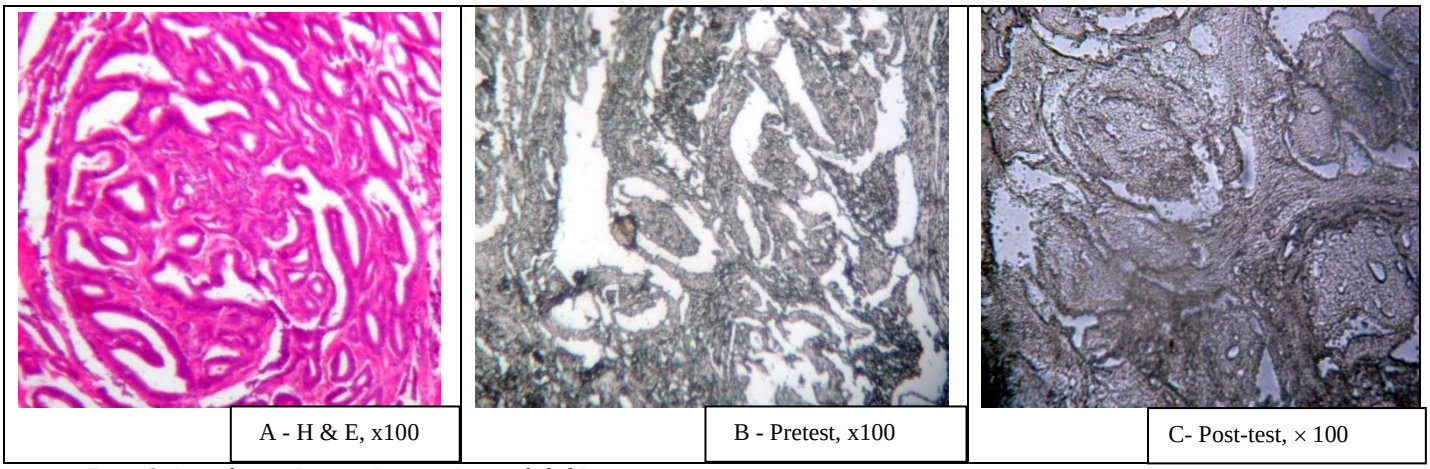

Figure 3. Premalignant Lesion – Prostatic Intraepithelial Lesion.
A - H & E Stain(x100)
B - Unstained deparaffinised section (before test x100).
C – Post SRCA test shows (x100) - focal/partial adhesion of RBCs at the epithelium.

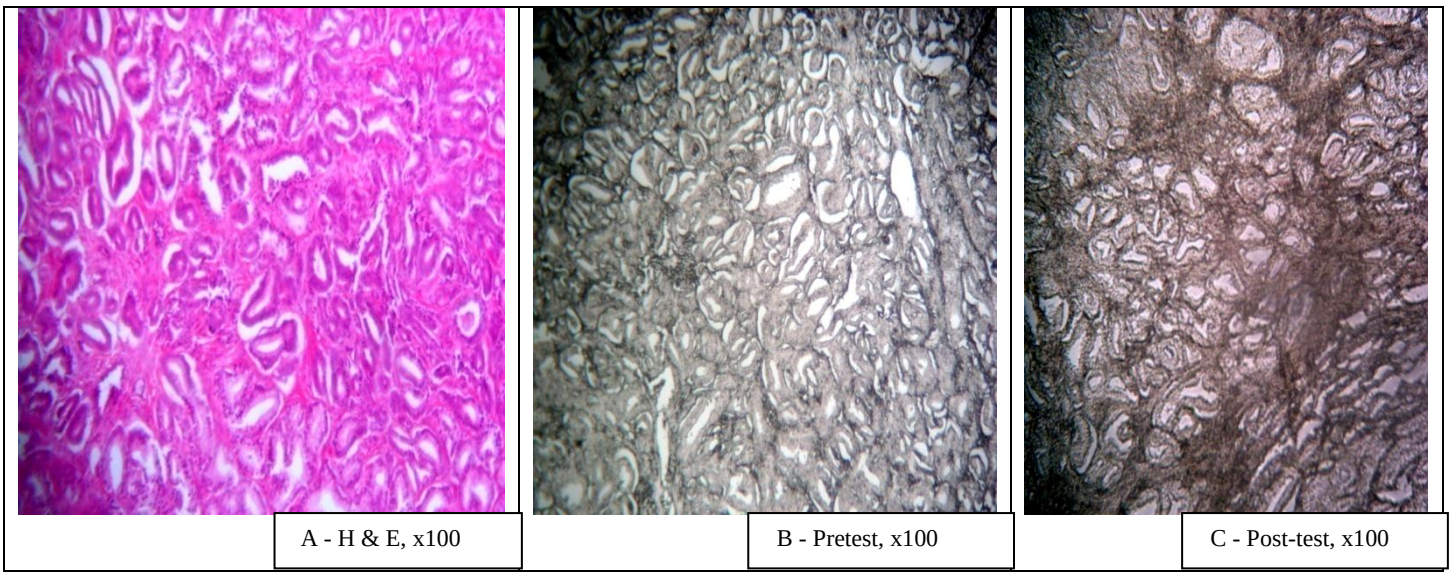

Figure 4. Malignant Lesion – Prostatic Carcinoma
A - H & E Stain(x100)
B - Unstained deparaffinised section (before test x100).
C – Post SRCA test shows (x100) - No adhesion of RBCs at the epithelium.

RESULTS

Specific Red Cell Adherence (SRCA) test in benign prostatic lesions:

Complete preservation of antigen (97.05%) was seen in 33 cases out of 34 Benign Prostatic Hyperplasia (BPH). The stroma in 34 cases of BPH was found to be negative except for an occasional streak of positivity in blood vessels which served as a built- in control. In 20 of the 34 cases, the prostatic stroma showed histologic evidence of prostatitis. In these areas SRCA test showed a patchy agglutination.

Specific Red Cell Adherence (SRCA) test in premalignant lesions:

Partial preservation of antigen (80%) was seen in 4 cases out of 5 premalignant lesions which includes prostatic atrophy and prostatic intraepithelial neoplasia (PIN). The stroma of 5 cases of premalignant lesions of prostate was found to be negative except for

an occasional streak of positivity in blood vessels.

Specific Cell Adherence (SRCA) test in malignant lesions:

Complete deletion of antigen (100%) was seen in 2 cases out of 2 malignant lesions which included primary adenocarcinoma of prostate. Scanty stroma separating the tumour acini showed occasional streak of agglutination in which RBC's were present in blood vessels.

The results of Specific Cell Adherence (SRCA) test on various prostatic lesions were tabulated as shown in Table No 1.

SRCA Test Results

SRCAT	Benign	Premalignant	malignant	Total
Ag. Complete preservation	33 (80.48%)	–	–	**33** (80.48%)
Ag. Partial preservation	1 (2.43%)	4 (9.75%)	–	5 (12.19%)
Ag. Complete deletion	–	1 (2.43%)	2 (4.87%)	**3** (7.31%)
Total	34 (82.92%)	5 (12.19%)	2 (4.87%)	41 (100%)

Table 1 Results of Specific red cell adherence test on various prostatic lesions.

DISCUSSION

The A, B and H antigens are complex carbohydrate structures found on glycoprotiens and glycolipids of the cell surface of erythrocytes and most of epithelial cells. Alles of the ABO gene code for glycosyltransferases that act on the precursor of H antigen. [(11)] Disappearance of antigen is due to absence of A or B transferase gene expression .As a result of loss of ABH isoantigens, red blood cells do not agglutinate giving a characteristic mixed field reaction. [(12)] Recent studies have shown that due to tumor development there is altered glycosylation, signal transduction and apoptosis of the cell surface proteins and lipids. [(13)] Alteration of ABH antigens in hematologic malignancy described a very weak A antigen expression on the red cells of a patient with acute myeloid leukemia who had previously shown normal A antigen expression. Loss of A, B, or H antigens from the surface of red blood cells is now recognized as a recurrent observation in hematologic malignancy. [(14, 15)] There are different studies which have shown the association of various cancerous lesions and loss of ABH isoantigens as per Gao S et al [(16)] and Hakomori S et al. [(17)]

Through this study, we have made an attempt to differentiate benign and malignant tumors by the use of above concept. The technique used was Specific Red Cell Adherence (SRCA) based on the principle of Mixed Cell Agglutination as mentioned previously by Kovarik S et al. [(2)]

In the present work, we used the loss of the expression of ABH antigens as a marker of differentiation.

As the expression of these antigens can be detected by monoclonal antibodies, they are a better objective marker of differentiation than the more commonly used subjective histologic assessment. The presence or absence of blood group antigens has been used to predict the clinical course of patients with superficial transitional cell carcinoma of the bladder. [(18,19)] The red-cell adherence test has been the most widely accepted method of antigen determination, but this technique has inherent weaknesses as improper tissue preservation and improper deparaffinisation there is false negative results.

Recently, the immunoperoxidase assay has been used to detect antigens on tumor cells. There are studies that have compared patients using the red-cell adherence and immunoperoxidase methods showed similar results (89%) when assessing the presence or absence of antigen. [(20)] The previous studies have suggested that in gastrointestinal and ovarian carcinomas the affected group-specific isoantigens may

possibly be related to a secretion product of cells forming the tumors. Previous workers had also reported somewhat similar findings and have suggested that a loss of isoantigens in malignant tumors may be an early indicator of cellular changes prerequisite for the ability to form metastatic lesions. [21-23]

CONCLUSION

SRCA test is an early indicator of risk of progression of malignancy at cellular level. It can be used as an alternative and adjuvant to immunoperoxidase and immunohistochemical staining. It is highly economical, cost-effective and reliable. It improves accuracy and reproducibility as it does not require extensive training. It helps in predicting recurrences and overall survival rate of patient clinically.

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