Differentiation of Tumors with Specific Red Cell Adherence (SRCA) test

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ABSTRACT

Introduction: The A & B blood group antigens present on various body tissues are lost following a malignant transformation. Through this study, we have made an attempt to differentiate benign & malignant tumors by the use of this concept. The technique used was Specific Red Cell Adherence (SRCA) based on the principle of Mixed Cell Agglutination on Fine-Needle Aspiration (FNA) samples.

Objective: Our aim was to perform the SRCA test on FNA samples of various human solid tumors and compare them with histopathology (Gold Standard).

Method: A total of 35 FNA samples of swellings suspected to be tumors were collected & the SRCA test was performed on them. The results were compared with histopathology.

Result: The sensitivity of SRCA test was found to be 88.23%; specificity was 72.22%; positive predictive value was 75%; negative predictive value was 86.66% ($p<0.05$).

Conclusion: SRCA test is an easy & cost-effective technique that can be used for differentiation of benign & malignant tumors on FNA samples.

Keywords: Specific Red Cell Adherence (SRCA), FNA (Fine-Needle Aspiration), Mixed Cell Agglutination

Introduction

The blood group antigens (A & B) are expressed in normal tissues of the human body other than red blood cells 1. Such antigens are also known as isoantigens. Some of these are lost in cells undergoing a malignant transformation. This property differs in different tissues 1-15. Majority of these isoantigens are expressed on epithelial
tissues & most human cancers tend to originate from these cells.
Solid tumors are a group of cells which have properties of invasiveness & metastasis, but at varying degrees. It is very difficult to correlate phenotypic characteristic of tumor cells with malignant biological behavior 1. The loss of A & B isoantigen expression has been shown in malignant epithelial neoplasms of the lung 1, 2, gastrointestinal tract 3, oral, cavity 4, cervix uteri 5, bladder 6-10, & prostate 11, thyroid neoplasm 12, epithelial ovarian cancer 13 & laryngeal cancer 14.In these tumor systems, the loss of isoantigens has been used to demonstrate transformation from benign to a malignant process 4; it's loss has also been used for prognosis 5, 9, 10. Studies on carcinomas of breast have shown no significant correlation between malignant transformation & loss of A & B isoantigen expression 15.

Fine needle aspiration cytology (FNAC) entails using a narrow gauge (25-22G) needle to collect a sample of a lesion for microscopic examination. It allows a minimally invasive, rapid diagnosis of tissue but does not preserve its histological architecture. In some cases this limits the ability to make a definitive diagnosis. As with any invasive procedure there are risks, and as with all diagnostic tests involving sampling and interpretation, important diagnoses can be missed. Immunohistochemical techniques are used in some centers, but are expensive. Hence there is a

need to develop cheap & affordable ways to diagnose malignant tumors to supplement routine morphological study. We have tried to do the same by determining A & B isoantigen loss on malignant tumor cells through an affordable & easy technique & using it as a diagnostic modality.

We used a technique based on the principle of "Mixed Agglutination of Erythrocytes" 4.
Mixed agglutination reaction was originally described as a result of investigations on the phenomenon of agglutination. Some of the workers described agglutination of two morphologically distinguishable cell types when they were mixed with antiserum to one of the cell types 4. If two different cells (for example, a red blood cell (RBC) & a tumor cell), are mixed with antiserum to one of the cell types (the RBC), the two cells will agglutinate if they share the same antigen (the A & B antigens in our case).

Retrospective studies have been done on paraffin-embedded sections of established malignant lesions to determine the loss of ABO isoantigens**.** The techniques used in these studies are variations of the mixed cell agglutination technique.

No study could be found demonstrating the use of this technique on Fine-Needle-Aspiration (FNA) smears.

The aim of this study is to develop a test using the principle of mixed cell agglutination to determine A & B isoantigens on tumor cells seen on "Fine-Needle-Aspiration (FNA)" samples of mass lesions or solid tumors & to determine whether it can be used as a diagnostic modality.

Material and Methods

1. Time-period : 6 months
2. Inclusion criteria:
   a. Patients with mass lesions suspected to be tumors
   b. Age group 12-60 years.

3. Exclusion criteria:
   a. Patients suffering from HIV & Hepatitis B
   b. Patients with suspected inflammatory swellings.
   c. Bloody FNA sample.

Methodology

- FNA samples were collected from patients when they were undergoing routine FNAC procedure after obtaining a valid written informed consent. A minimum of two slides were prepared extra for every patient.
- The slides were immediately fixed in 95 % ethyl alcohol.
- The slides then were tested for determination of A & B isoantigens by a technique called "Specific Red Cell Adherence Test (SRCA)" by the technique of Kovarik et al 4. The procedure is described in the subsequent steps.
- The first slide was treated with antibodies for blood group A (using the anti-sera routinely used for blood-group cross matching) & incubated in a moist chamber at room temperature for a period of 20 minutes.
- The excess antiserum was then washed of using normal saline.
- The slide was then treated with 5% solution of Red Blood Cells (RBCs) of group A.
- The same procedure was repeated for the second slide but this time the antiserum for blood group B & 5% solution of RBCs of the B group was used.
- The slides were then kept in a vertical position in 95% ethyl alcohol for a period of 15 minutes. This ensured that the unattached RBCs & other RBC clumps settle down & simultaneous fixing took place for staining.
- Then, the routine Hematoxylin & Eosin (H&E) staining was done, and the slides were examined under the microscope by a qualified pathologist.

- More than or equal to five red blood cells adhering to a tumor cell was considered a positive result.
- The results were compared to routine morphological examination & histopathological results of the same case.

Results

The technique was used on 35 samples of human solid tumors. Of them, 14 were breast tumors, 5 were lipomas, 6 were lymph node swellings, 3 were ganglion cysts, 3 were metastatic tumors & 4 others (1 benign spindle cell tumor, 1 epidermal cyst, 1 pleomorphic adenoma, 1 neurofibroma) Figure 1. The sensitivity of the test was found to be 88.23%, specificity 72.22%, positive predictive value 75%, negative predictive value 86.66% & accuracy was 80%. The likelihood ratio of a positive test was 3.17 & negative test was 0.16 Table 1. Significance was calculated by the Yates chi-square test, corrected for continuity (degree of freedom was one). The p value was calculated to be 0.0011 ($p<0.05$).

Discussion

Our study is a preliminary one to attempt the use of the SRCA technique on FNA samples. No study could be found demonstrating the use of this test on FNA samples. he SRCA technique based on mixed cell agglutination was first described by Kovarik et al 4. The malignant transformation of lung 2, cervix 5, bladder 6-10, prostate 11, gastrointestinal tract 3, thyroid 12, laryngeal cancer 14 & epithelial ovarian cancer 13 correlate strongly with A & B isoantigen loss, most of these tumors being epithelial neoplasms. Our study does not study the tumors individually.

Conclusion

SRCA technique offers the advantage of being highly economical, as compared to immunohistochemical staining. With the elimination of the need for a detailed morphological

study, it also does not require extensive training. This not only improves accuracy but also reproducibility. It has the potential of becoming a reliable & cost-effective alternative to other techniques of FNAC. More studies are needed to study individual tumors by the use of this technique & also to develop a standard statistical method for analysing the results.

Limitations:

1. Past studies demonstrating the use SRCA test
on histopathology slides demonstrate varied loss of A & B isoantigens in malignant transformation of different tissues.
2. Our study does not consider the tumors individually.
3. The test fails to give results on a bloody sample.
4. The blood group status of the patient was not considered.

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Conflict of Interest: None declared.

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Table 1: Table showing the parameters of the SRCA test

Parameters	**Value**	**95% Confidence Interval**	
		Lower limit	**Upper limit**
Sensitivity	0.8823	0.6225	0.9793
Specificity	0.7222	0.4640	0.8928
Positive Predictive Value	0.7500	0.5058	0.9040
Negative Predictive Value	0.8666	0.5838	0.9765
Likelihood ratio	**Value**		
Positive test	3.1764	1.4783	6.8252
Negative test	0.1628	0.0424	0.6244

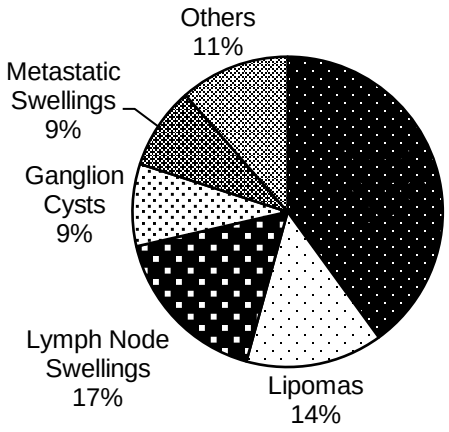

Figure 1: Figure showing the type of tumors considered in the study