|| ISSN(online): 2589-8698 || ISSN(print): 2589-868X ||
International Journal of Medical and Biomedical Studies
Available Online at www.ijmbs.info
NLM (National Library of Medicine ID: 101738825)
Index Copernicus Value 2019: 79.34
Volume 5, Issue 4; April: 2021; Page No. 84-95

Original Research Article

STUDY OF A, B, O (H) ISOANTIGENS IN PARAFFIN SECTIONS OF MALIGNENT TUMORS

Dr. Shyam Sunder Bajaj

Associate Professor, Department of Pathology, Amaltas Institute of Medical Sciences, Dewas (M.P.).

Article Info: Received 15 February 2021; Accepted 09 April 2021
DOI: https://doi.org/10.32553/ijmbs.v5i4.1845
Corresponding author: Dr. Shyam Sunder Bajaj
Conflict of interest: No conflict of interest.

Abstract

The present study entitled, Study of A,B,O (H0 Isoantigens in paraffin sections of malignant tumors was conducted among the patients at Amaltas Institute of Medical Sciences, Dewas. Patients coming to any clinical department with tumors during the period ware randomly selected for the study . After complete history and thorough clinical examination, surgery of tumors was done and tissues were sent for histopathology examination.

Introduction:

The agglutination of red cells of one species with serum from other species was observed by Landois as early as 1875. This phenomenon suggested that agglutination of red blood cell was due to antigens on their surface uniting with the antibodies in the serum.

Antigens identical to A,B and O(H) blood group isoantigens were demonstrable in some tissues and body fluids. This was reported by coombs et al (1956) ; Kovarik et al (1968) : Dayasi et al (1973) . By high sensitive and specific techniques these blood group isoantigens could serve as trace antigens for the study of change in malignant transformation Kovarik et al, (1968).

Search for a simple and easily performed technique was rewarded in development and perfection of mixed cell agglutination reaction (MCAR) by coombs. This test apart from being simple, was easily performed requiring no costly laboratory apparatus; gave comparable results to immunofluorescence technique.

MCAR proved very useful to pathologists engaged in immunologic study of tumor pathology and behaviour of group specific substances in tissues.

There are a lot of objections on the nomenclature of the test i.e. mixed cell agglutination reaction name originally coined by coombs. Davidsohn prefers to call it specific red cell Adherence test because it fits better the actual appearance of tissues though the designation MCAR is still applicable to reaction between erythrocytes in the lumina of blood vessels and in extra vasations and the indicator erythrocytes.

By using specific red cell adherence test the isoantigens , A,B, and O were not usually demonstrable in malignant tumours, but they were easily observed in normal and benign tissues as reported by coomb ser al (1956) ; Davidsohn et al (1968, 1973) Complete absence of blood group antigens was interpreted as a manifestation of functional dedifferentialtion in cancer analogous to morphological dedifferentiation of anaplasia.

It was well established that during carcinogenesis there is emergence of new antigens which were distinct form the normal histocompatibillty antigens and were recognised as tumor specific antigens. During this process there was also deletion of some normal antigens as was suggested by green as early as 1954. In the normal tissues isoantigens A,B and O (H) have been easily identified which are different from tumour specific antigens (Dayasi et al , 1973) So it was decided to probe into the mechanism of non – demonstrability of blood group isoantigens in malignancy. It might be due to inability to produce, store or destruction of isoantigens by cancerous transformation of masking of isoantigens (Kavarik, 1968).

It was also known that blocking factors in serum, presumably specific antibodies to tumor transplantation, specific antigens or immune complexes may be present on the surface of the tumor cells or reactive lymphocytes (Baldwin and Robins, 1976) These blocking factors or immune complexes might be responsible for the escape of immunological on slaught of the tumour cells by specific cytotoxic lymphocytes or killer cells.

It was presumed that blocking factors or immune complexes deposited on the surface of the tumor cells might be masking the antigenic sites or determinents for blood group isoantigens normally present on the cell membranes.

So many workers have tried to find out suitable techniques or methods to remove immune complexes present on the surface of the cells. Alexender (1973) used several repeated washing and centirifugalisation of suspension of lymphocytes. But this was not possible on paraffin sections.

The other method i.e. use of a mild detergent was considered worth trying and was found suitable (Cyril et al. (1978)

In a recent study by Trikha, N, Monga, J.N. and associates (1978) a weak solution of detergent was used to remove the

blocking factors from tumor cell surface. In their study the detergent treated paraffin sections, revealed a strongly positive SRCA test, which were originally consistently negative for blood group isoantigens in the same untreated sections of malignant tumors.

In our study we also used a weak solution of detergent to remove the blocking factors from tumour cell surface.

So our present study was undertaken to document the observation of demonstrability of blood group isoantigens in paraffin section before and after the use of detergent.

The results were significant enough to throw considerable light on the variable mechanism of failure to demonstrate blood group isoantigens in malignant cells.

REVIEW OF LITERATURE

Distribution of blood group substances in tissues

Earlier it was known that ABH substances occur in human tissues and secretions in 2 forms – 1 water soluble, 2 alcohol soluble and that persons with these substances in saliva (secretors) have more water soluble substances in their tissues than those lacking the substance in saliva (non- secretors) . But immunoflourescence staining has revealed the ABH substances are found in the cell membranes of all vascular endothelial cells and certain epithelial cells. The membrane antigens are alcohol soluble and occur in secretors and non-secretors alike. The positive epithelia are stratified and pseudostratified and include the skin, tongue, oesophagus, lower genito - urinary tract and uterine cervix.

The test holds promise – (1) to be valuable in diagnosis of early carcinoma in tissues that normally contain the three isoantigens and in prognosis of advanced carcinoma, (11) to reduce the need of radical surgery in carcinoma in situ of cervix.

He found that in carcinoma the intensity of reactions was inversely related to the degree of anaplasia.

They found 3 types of results of SRCA – (I) positive reaction, (II) negative reaction, and (III) incomplete or patchy reaction.

The incomplete reaction is interpreted as result of progressive loss of antigen which parallels the progressive anaplasia of carcinoma.

Convincing examples of parallelism between progression of carcinoma and the progressive loss of antigens are – (I) carcinoma in situ in which incomplete adherence test was more frequent than in any other stage of carcinoma, (II) metastatic carcinomas of all varieties in which the loss of antigen evinced by negative adherence test was the rule in lung and cervix , 88 % of carcinoma of stomach and 84% in pancreatic carcinomas.

The negative tests in most metastatic carcinomas and in almost all distant metastases permits the conclusion that the loss of 3 isoantigens precedes the formation of metastases.

They came to the conclusion that-

- They loss of three antigens A, B and H is an early apparently the earliest presently (1972) available indicator of carcinoma.
- In borderline cases in controversy of benign or malignant, loss of isoantigens favour the diagnosis of carcinoma.]
- A positive reaction indicates presence of isoantignes and makes the diagnosis of carcinoma less likely.
- A negative reaction reaction in a primary carcinoma suggests the possibility or probability of or even presence of metastases.
- A positive reaction in a primary carcinoma makes the presence of metastases extremely unlikely.
- The adherence test may be useful in early diagnosis and prognosis of carcinoma of organs and tissue that normally contain the isoantigens.
- The need of radical surgery in carcinoma in situ of the cervix may be eliminated in cases in which the presence of A,B,H antigens is established by positive SRCA test.

Dayasi and Aikat (1973) studied 45 malignant and non-malignant tissues of ear, nose and throat regions. These tumours were from oesophagus, larynx ,nasal cavity , maxillary antrum , nasopharynx, oral cavity , oropharynx and face, Oral cavity and oropharynx included tongue and tonsils . Majority of the tumours chosen had squamous cell epithelium and hence tumours were mostly squamus cell carcinomas. The next frequent variety was Adenocarcinoma.

Twenty-one tumours were SRCA negative and indicate a deletion of A,B,H isoantigens from them , there by indication advance malignancy . These tumours clinically had metastasis the results were more or less uniform in each group.

Specific red cell adherence test promises to be useful in early diagnosis and prognosis of squamous cell carcinoma of cervix.

The hypothesis to come for failure to demonstrate blood group isoantigens in cancer (Kovarik et al , 1968) suggested that the failure to demonstrate blood group isoantigens in cancer might be due to the following factors:

1. Inability to produce blood group isoantigens.
2. Inability to store.
3. Destruction of isoantigens by cancerous transformation.
4. Masking of isoantigens.

It has also been shown that serum contains blocking factor which under appropriate situation may inhibit the cytotoxic effect of lymphocytes. There has been several studies to find out the role of blocking factors interfering with immunological rejection of tumour.

In a recent study by Cyril, J. et al (1978) immunochemical analysis of inner and outer membranes of Escherichia coli by crossed immune-electrophoresis was done. The non -

ionic detergent Triition-x-100 was used and proved to be extremely useful in the extraction of bacterial membrane antigens, and in their subsequent analysis by crossed immune-electrophoresis. Based on this study, it was thought to remove the blocking factors from the tumour cell surface by washing the paraffin section in a weak solution of detergent.

In another study, Trikha, N. and Monga, J.N. et al (1978) used a weak detergent solution to remove the blocking factors from tumour cell surface. In their study the detergent treated paraffin section revealed strongly positive MCAR or SRCA tests, which were originally consistently negative fro blood group isoantigens in the same untreated section of malignant tumors.

In our study also we used a weak solution of detergent to remove the blocking factors from the tumor cell surface.

AIMS AND OBJECTS

The aims of our present study were –

1. To study the presence of isoantigens A, B, and O (H) in:

I. Normal tissues (inflammatory lesions).

II. In benign lesion.

III. In malignant tissues.

It has been observed by several workers that isoantigens A, B, and O (H) are reduced or absent in cancerous tissues and are present in normal and benign tissues. It is thought that it might be due to inability to produce . store or destruction of isoantigens by cancerous transformation or masking of isoantigens (Kovarik et al , 1968).

2. So our further aim was to probe into the likely mechanism of failure to demonstrate blood group isoantigens in malignant tumours.

MATERIALS AND METHODS

The present study was undertaken to demonstrate A, B and O (H) isoantigens in normal tissues, benign lesions and in malignant neoplasms, before and after treatment with a weak (1%) solution of detergent. Total number of cases studied were 105.

The material was selected from the biopsies obtained in Histopathology section of Amaltas Institute of Medical Sciences, Dewas (M.P.) from October 2019 to September 2020.

The blood groups of the patients, whose biopsises were studied, were done.

The study was divided into two groups .

1) Control Group

2) Test Group

(1) Control group

(a) In this group total 25 cases were studied. Distribution of types tissues studied in this group are shown in Table I (a).

Table – I (a)

S. No.	Tissues	Histopathology Report	No. of cases
1	Cervix	Endocervicitis	8
2	Skin	Skin unremarkable	4
3	Buccal mucosa	- Nonspecific inflammation	1
		- Hyperkeratotio lesion	1
4	Tongue	-Nonspecific inflammation	1
		-Hyperkeratotion lesion	1
5	Rectal mucosa	Nonspecific inflammation	1
6	Colonic	Colonic mucosa unremarkable	1
7	Prostate	Benign hyperplasia of prostate	4
8	Breast	Fibroadenoma of breast	3

(b) Out of 80 malignant tumors studied , normal tissue was present in 28 cases in the same sections of carcinoma. The normal tissue served as built in control.

(2) This group included 80 cases of malignancy. Distribution of different tissues studied in the group are shown in Table – II (a).

Table – II (a)

S. No.	Tissues	Histopathology Report	No. of cases
1	Cervix	Squamous cell carcinoma cervix	35
2	Cheek	Squamous cell carcinoma Cheek	12
3	Larynx	Squamous cell carcinoma Larynx	8
4	Oesophagus	Squamous cell carcinoma Oesophagus	4
5	Tongue	Squamous cell carcinoma Tongue	5
6	Penis	Squamous cell carcinoma Penis	3
7	Breast	Duct carcinoma breast	7
8	Prostate	Adenocarcinoma prostate	3
9	Sygmoid colon	Adenocarcinoma sygmoid colon	1
10	Rectum	Adenocarcinoma rectum	2

(b) 56 cases of malignant tissues from above group were also studied for SRCA after treatment with a weak solution of detergent.

TECHNIQUE OF DEMONSTRATION OF A,B, AND O (H)

ISOANTIGENS IN TISSUE SECTION

We used specific red cell adherence test (SRCA) (Original method of Davidson, I , (1969) modified by Deyasi , S.K. , Aikat, B.K. and associated (1973) for demonstration of isoantigens A, B and O (H).

Material requited

1) Tissuess: Paraffin sections of 5-6 microns thickness mounted on slides were used.
2) Antisera: Grouping antisera , Anti – A , Anti- B , Anti – AB in titres of 1 in 60 were used . Extract of Ulex europeus with tissue of 1 in 60 for group 'O' (Associated Laboratories Pvt. Ltd: Bombay) was used.
3) Indicator Erythrocytes: Erythrocytes of group A, B, AB and O washed in isotonic saline in 5 % suspension.
'O' group erythrocytes were treated with 0.1% solution of papaine oystine for ½ hour before the 5 % supesnion was made.

STEPS OF SPECIFIC RED CELL ADHERENCE TEST

(1) Four paraffin sections of each type of tissue were taken and were deparffinzed. For this – the sections were first placed in an incubator at temperature of 60^{o}C for about 15 minutes . Then they were immediately put in xylene for about ½ hour . Then the xylene was removed by putting sections in absolute alcohol for about 5 minutes. Then the sections were washed in tap water and then rinsed in normal saline. Then each slide was placed in a separate glass Petridishes with moist filter paper. Blood group antigen was carefully marked on each slide and on the outer surface of the bottom of the petridish for easy recognition.

(2) Three grouping antisera and Anti –H (extract of Ulex Eutopeus) were poured on the slides with a fine pipette to cover only the tissue section and kept for 20 minutes in the moist petridish.

(3) The antiesera were removed by tilting the slides and were washed with three changes of isotonic normal saline over a period of about 6 minutes.

(4) Each section was then covered with the corresponding indicator erythrocytes suspension for about 15 minutes.

(5) Another four glass petridishes similarly marked were filled with little isotonic saline. Each slide was then briskly turned upside down in these perridishes and placed on 2 parallel supporting sticks of wood or plasticin. The lower surfaces of the slides just touched the upper surface of isotonic saline.

(6) Within 10 minutes the non-reacting erythrocytes sank to bottom of the petridish.

The slides were then slid across to a clear area of saline. The petridish was put gently on the plateform of a microscope. The sections were examined under low power of the microscope through the thickness of the slide with tissue section remaining on the lower surface touching the normal saline .The results were recorded as follows:

1. Positive reaction i.e. presence of isoantigens in the tissue section.
2. Negative reaction i.e. absence of isoantigens in tissues sections.
3. Incomplete or patchy reaction i.e. presence of the isoantigens in some areas and its absence in other areas in the same section

The positive reaction is indicated by the adherence of the indicator erythrocytes to the epithelium and the capillary endothelium of blood vessels.

The specificity of SRCA was controlled by –

1. Putting up control with A group of tissues to react with Anti –B and Anti-H (Ulex Europeus Extract).
2. Putting up control with a group B tissues to reaction with Anti – A and Anti –H (Ulex Europeus Extract).
3. Use of non - isologous erythrocytes.
4. Difference in reactivity of normal and cancerous tissues adjacent to each other in the same section when available.

The test is basically a " sandwich reaction " where tissue is the bottom layer , the erythrocytes are the top layer and specific antisera – the middle layer, that holds the top and bottom layers together . If the indicator erythrocytes of the top layer remain attached, the conclusion is that the tissue cells in the area of reaction contain the same A, B or O antigen that is present in indicator erythrocytes.

TECHNIQUE FOR PRIOR TREATMENT OF SECTIONS WITH DETERGENT BEFORE

S.R.C.A.

(Technique was obtained by personal communication from Mrs. N. Trikha and J. N. Monga, Gwalior – study is under publication

The malignant tumours which initially showed uniformly negative SRCA were treated with detergent solution and SRCA test was done.

This group include 56 cases. The types of malignant tissues studied are shown in table – II(a).

SRCA was done simultaneously on two paraffin section, one without treating with detergent and other after treatment with detergent.

Technique

1. For paraffin sections of each type of tissue were taken and deparaffinized by routine standard technique . Then slides were put in coplin's jar having 1% detergent solution for one hour (prepared by dissolving 1gm. Of surf powder in 100 cc. of distilled water.
2. The slides were washed in another coplin jar for one hour under running tap water to remove all traces of detergent.
3. Then simultaneously another four section were deparaffinised SRCA was done with detergent treated and non – detergent treated sections simultaneously and the results were noted as described earlier.

OBSERVATION

A study of A,B and O (H) is isoantigens in normal tissues, benign lesions and malignant tissues was done in 105 cased by specific red cell adherence test.

Three types of reactions were observed-

1. Positive reaction (+) i.e. present of isoantigens in the tissue section.
2. Negative reaction (-) i.e. absent of isoantigens in tissues section.
3. Incomplete or patchy reaction (+) i.e. presence of isoantigens in some areas and its absence in other areas in the same section.

Observation were made as follows;

Distribution of isoantigens A,B, AB and O (H) antigens in –

1. Normal tissues.
2. Benign lesions.
3. Malignant tissues.
4. Malignant tissues after treatment with detergent.

Study of A,B & O (H) isoantigens in normal (non – malignant) tissues

In this group we studied 18 cases of normal (non – malignant) tissues. These included cases of endocervicitis, normal skin, non – specific inflammation of buccal mucosa, tongue and rectal mucosa hyperkeratotic lesions of buccal mucasa - and tongue and normal colonic mucosa .The observations of SRCA test in normal (non-malignant) tissues are shown in Table-I.

Table 1: Result of specific red cell adherence test in normal (non-malignant) tissues

S.No	Tissue	Name	Age/Sex	Biopsy No.	Blood Group	Histopathology Report	Parenchymatous Tissue	stroma	Blood vessels	Gland Secretions
1	Cervix	S.B.	40/F	998/19	B	Endocervicitis	+	-	+	+
2	Cervix	P.B.	35/F	1912/19	B	Endocervicitis	+	-	+	+
3	Cervix	K.B.	45/F	1433/19	B	Endocervicitis	+	-	+ -	-
4	Cervix	D.B.	45/F	1829/19	A	Endocervicitis	+	-	+	+ -
5	Cervix	L.B.	50/F	1379/19	B	Endocervicitis	+ -	-	+	+
6	Cervix	D.B.	50/F	2015/19	O	Endocervicitis	+	-	+	-
7	Cervix	S.B.	45/F	700/19	A	Endocervicitis	+	-	+	+
8	Cervix	S.B.	50/F	719/19	B	Endocervicitis	+	-	+	-
9	Rectal mucosa	P.B.	20/F	584/19	A	Non specific inflammation rectal mucosa	+	-	+	+
10	Colonic Mucosa	L.B.	40/F	1686/19	B	Colonic mucosa unremarkable	+	-	+	+
11	Skin	B.L.	25/M	A1/19	B	Skin unremarkable	+	-	+	-
12	Skin	J.K.	20/M	A2/19	B	Skin unremarkable	+	-	+	-
13	Skin	N.K.	30/M	A3/19	A	Skin unremarkable	+	-	+	-
14	Skin	L.S.	40/M	A4/79	B	Skin unremarkable	+	-	+	-
15	Buccal Mucosa	S.B.	65/F	146/19	A	Non specific inflammation	+	+ -	+	
16	Buccal Mucosa	R.L.	60/M	574/19	B	Hyperkeratatic lesion	+	-	+	
17	Tongue	N.D.	47/M	148/19	O	Non specific inflammation	+	-	+	
18	Tongue	G.S.	23/M	1113/19	B	Hyperkeratatic lesion	+	-	+ -	

Result of SRCA test in normal (non-malignant) tissues

In our study results of SRCA test in normal tissues was as follow:

To summarize with SRCA test was positive in parenchymatous tissues and negative in stroma in all the normal (non-malignant) tissues i.e isoantigens A, B O (H) were present in parenchymatous tissues, while they were absent in stroma in all the cases.

In case of carcinoma cervix SRCA was positive in super ficial layer of stratified squamous epithelium, in mucous glands and mucous secretions.

In squamous cell carcinoma of cheek, larynx, SRCA test was positive (+) in stratified squamous epithelium, mucous glands, and lining of blood vessels. The stratified squamous epithelium of various tumours showed the same pattern of SRCA as observed in the stratified squamous epithelium of skin.

Study of isoantigens A , B, o (H) in benign lesions

Four cases of benign hyperplasia of prostate and 3 cases of Fibroadenoma breast were studied for present of A , B, and O (H) isoantigens by SRCA test . Observations are shown in table – II.

Table 2: SRCA in benign lesions

S.No	Tissue	Name	Biopsy No.	Blood Group	Parencym. tissue	Blood vessels	stroma	Secretion in glands	Parencym. tissue	Blood vessels	stroma	Secretion in glands
1	BPH	V.S.	1247/19	B	+	+	-	+	++	+	-	+
2	BPH	C.	1463/19	A	+	+	-	+	+	++	-	+
3	BPH	B.D.	2235/19	AB	+	+	-	-	++	++	-	+
4	BPH	S.R.	2114/19	A	+	+	-	+	+	+	-	+
5	Fibroadenoma breast	K.	1245/19	B	+	+	-	+	+	+	-	+
6	Fibroadenoma breast	U.	614/19	A	+	+	-	+	+	+	-	+
7	Fibroadenoma breast		1248/19	O	+	+	-	+ -	+	+	-	+

Results of SRACA test in benign lesions before and after treatment

1. Benign hyperplasia of prostate: Four cases of benign hyperplasia of prostate were studied. Before treatment with detergent SRCA test was positive (+) in parenchymatous tissue (lining of acini) and blood vessels in all the 4 cases (100%). It was found to be negative (-) in stroma in all the 4 cases (100%). SRCA test was positive in gland secretions in 3 cases (75%) while it was negative in one case (25%).

After treatment with detergent SRCA test was found to be positive in parenchymatous tissue and blood vessels in all the 4 cases (100%). Also there was increase in the intensity of SRCA in parenchymatous tissue and blood vessels in 2 cases (50%) . SRCA test was also positive in gland secretion in all the 4 cases (100%), while it was negative (-) in stroma in all cases (100%).

2. Fibroadenoma of breast : total 3 cases were studied. Before treatment with detergent SRCA test was positive (+) in parenchymatous tissue and blood vessels in all the 3 cases (100%) while it was negative (-) in stroma in all the case (100%) . SRCA test was positive in gland secretions in 2 case and it was patchy (+)in gland secretions in one case.

After treatment with detergent the results were found to be same. So in cases of benign lesions isoantigens A,B, O (H) were present parenchymatous tissues and were absent in stroma, even after treatment with detergent.

Study of isoantigens A,B, O(H) in malignant tissues

Total 80 malignant tissues were studied for the presence of isoantigens A,B, and O (H)

By SRCA test . The types of tissues studied included carcinoma of cervix , cheek, larynx , tongue , oesophagus , penis, breast, prostate and carcinoma of large bowel.

The observation are shown in table – III

Table –III SRCA in Malignant Tissues

S.No	Tissue	Name	Age in years	Sex	Slide No.	Blood Group	Histopathology report	T. N.M. Classification	Stage
1	Cervix	P.K.	63	F	1849/20	B	Inv . W.D. Sq. cell ca.cx.	$T_3N_1M_0$	III
2	Cervix	R.K.	75	F	2190/20	A	Inv . P.D. Sq. cell ca.cx.	$T_3N_4M_0$	IV
3	Cervix	P.B.	45	F	2703/20	A	Inv . P.D. Sq. cell ca.cx.	$T_2N_1M_0$	III
4	Cervix	K.B	45	F	2702/20	B	P.D. Sq. cell ca.cx.	$T_2N_1M_0$	III
5	Cervix	J.B	40	F	4418/20	A	W.D. Sq. cell ca.cx.	$T_4N_1M_0$	IV
6	Cervix	R.K.	40	F	5813/20	B	Anaplastic Sq. cell ca.cx.	$T_2N_1M_0$	III
7	Cervix	S.B.	40	F	2146/20	O	P.D. Sq. cell ca.cx.	$T_2N_0M_0$	IIa
8	Cervix	B.B.	45	F	2977/20	O	Undiff. Sq. cell ca.cx.	$T_3N_1M_0$	III
9	Cervix	B.B	50	F	2437/20	B	P.D. Sq. cell ca.cx.	$T_2N_1M_0$	III
10	Cervix	S.B.	50	F	5765/20	AB	P.D. Sq. cell ca.cx.	$T_2N_1M_0$	III
11	Cervix	B.B.	50	F	5973/20	B	P.D. Sq. cell ca.cx.	$T_2N_1M_0$	III
12	Cervix	T.B.	40	F	3122/20	A	P.D. Sq. cell ca.cx.	$T_2N_1M_0$	III
13	Cervix	K.B.	48	F	2322/20	AB	W.D. Sq. cell ca.cx.	$T_2N_1M_0$	IIb
14	Cervix	S.B.	50	F	2747/20	O	P.D. Sq. cell ca.cx.	$T_2N_0M_0$	IIb
15	Cervix	D.B.	55	F	2639/20	O	W.D. Sq. cell ca.cx.	$T_2N_0M_0$	III
16	Cervix	S.K.	50	F	557/20	BD	P.D. Sq. cell ca.cx.	$T_3N_1M_0$	III
17	Cervix	M.B.	40	F	2828/20	O	W.D. Sq. cell ca.cx.	$T_2N_1M_0$	III
18	Cervix	G.B.	50	F	3129/20	O	W.D. Sq. cell ca.cx.	$T_2N_1M_0$	III
19	Cervix	B.B.	60	F	3023/20	O	W.D. Sq. cell ca.cx.	$T_3N_1M_0$	III
20	Cervix	C.K.	25	F	1043/20	A	W.D. Sq. cell ca.cx.	$T_2N_1M_0$	IIa
21	Cervix	K.B.	60	F	1456/20	B	W.D. Sq. cell ca.cx.	$T_2N_1M_0$	III
22	Cervix	G.B.	30	F	2640/20	A	W.D. Sq. cell ca.cx.	$T_2N_1M_0$	III
23	Cervix	N.B.	45	F	3042/20	B	M.D. Sq. cell ca.cx.	$T_2N_1M_0$	III

24	Cervix	P.B.	70	F	49/20	O	W.D. Sq. cell ca.cx.	$T_2N_1M_0$	III
25	Cervix	R.B.	50	F	143/20	O	W.D. Sq. cell ca.cx.	$T_3N_1M_0$	III
26	Cervix	K.D.	35	F	329/20	B	W.D. Sq. cell ca.cx.	$T_1N_1M_0$	Ib
27	Cervix	B.B.	85	F	1004/20	B	P.D. Sq. cell ca.cx.	$T_1N_0M_0$	Ib
28	Cervix	P.B.	35	F	1273/20	A	W.B. Sq. cell ca.cx.	$T_2N_0M_0$	IIb
29	Cervix	A.R.	30	F	528/20	AB	W.D. Sq. cell ca.cx.	$T_2N_0M_0$	Ib
30	Cervix	J.B.	65	F	1355/20	A	W.D. Sq. cell ca.cx.	$T_2N_0M_0$	IIb
31	Cervix	S.B.	50	F	527/20	A	W.D. Sq. cell ca.cx.	$T_3N_1M_0$	III
32	Cervix	U.B.	55	F	690/20	B	P.D. Sq. cell ca.cx.	$T_2N_0M_0$	IIb
33	Cervix	T.B.	40	F	740/20	O	W.D. Sq. cell ca.cx.	$T_2N_0M_0$	Ib
34	Cervix	S.B.	40	F	1573/20	A	M.D. Sq. cell ca.cx.	$T_1N_0M_0$	Ib
35	Cervix	R.B.	40	F	1574/20	O	W.D. Sq. cell ca.cx.	$T_2N_0M_0$	IIb
36	Cheek	A.B.	60	F	3008/20	B	W.D. Sq. cell ca.Buccal mucosa	$T_4N_0M_0$	IV
37	Cheek	C.	50	M	1677/20	B	W.D. Sq. cell ca.Buccal mucosa	$T_2N_0M_0$	II
38	Cheek	M.L.	60	M	1239/20	O	W.D. Sq. cell ca.Buccal mucosa	$T_4N_1M_0$	IV
39	Cheek	U.	35	M	4453/20	O	W.D. Sq. cell ca.Buccal mucosa	$T_2N_1M_0$	III
40	Cheek	M.C.	50	M	2452/20	O	W.D. Sq. cell ca.Buccal mucosa	$T_2N_3M_0$	IV
41	Cheek	K.S.	50	M	2153/20	B	W.D. Sq. cell ca.Buccal mucosa	$T_2N_1M_0$	II
42	Cheek	S.K.	60	M	5243/20	B	W.D. Sq. cell ca.Buccal mucosa	$T_2N_1M_0$	III
43	Cheek	R.	76	F	5170/20	O	W.D. Sq. cell ca.Buccal mucosa	$T_2N_1M_0$	III
44	Cheek	K.	50	F	2538/20	O	W.D. Sq. cell ca.Buccal mucosa	$T_2N_1M_0$	III
45	Cheek	M.S.	40	M	2249/20	B	P.D. Sq. cell ca.Buccal mucosa	$T_2N_1M_0$	III
46	Cheek	C.B.	40	F	1701/20	O	P.D. Sq. cell ca.Buccal mucosa	$T_3N_0M_0$	IV
47	Cheek	T.R.	50	M	866/20	A	W.D. Sq. cell ca.Cheek	$T_3N_0M_0$	III
48	Larynx	F.	45	M	1959/20	B	P.D. Sq. cell caLarynx	$T_1N_1M_0$	III
49	Larynx	H.C.	35	M	3310/20	B	W.D. Sq. cell caLarynx.	$T_1N_1M_0$	III
50	Larynx	H.	50	M	2223/20	B	Anaplastic ca. Larynx	$T_2N_1M_0$	III
51	Larynx	K.	40	F	5529/20	O	W.D. Sq. cell ca Larynx.	$T_1N_1M_0$	III
52	Larynx	K.	20	M	3125/20	O	W.D. Sq. cell ca Larynx.	$T_1N_1M_0$	I
53	Larynx	S.	40	F	709/20	A	W.D. Sq. cell ca Larynx.	$T_1N_1M_0$	I
54	Larynx	R.K.	50	M	3997/20	B	W.D. Sq. cell ca Larynx.	$T_1N_1M_0$	III
55	Larynx	N.	58	M	1053/20	B	W.D. Sq. cell ca Larynx.	$T_1N_1M_0$	III
56	Tongue	B.L.	60	M	6229/20	B	W.D. Sq. cell tongue	$T_4N_1M_0$	IV
57	Tongue	S.	50	M	2117/20	B	W.D. Sq. cell tongue	$T_2N_1M_0$	III
58	Tongue	R.	55	F	2120/20	A	W.D. Sq. cell tongue	$T_2N_0M_0$	II
59	Lip	H.N.	47	M	5570/20	O	W.D. Sq. cell ca.cx. lip	$T_1N_0M_0$	I
60	Oesophagus	D.N.	50	M	2076/20	O	W.D. Sq. cell ca. Oesophagus	$T_1N_1M_0$	II
61	Oesophagus	B.L.	50	M	1898/20	B	W.D. Sq. cell ca. Oesophagus	$T_1N_1M_0$	I
62	Oesophagus	J.B.	48	F	2004/20	B	W.D. Sq. cell ca. Oesophagus	$T_2N_0M_0$	I
63	Oesophagus	S.M.	61	M	5324/20	O	W.D. Sq. cell ca. Oesophagus	$T_1N_0M_0$	I
64	Tongue	N.L.	80	M	16/20	B	P.D. Sq. cell ca tongue	$T_2N_3M_0$	IV
65	Penis	B?	50	M	2079/20	B	W.D. Sq. cell ca Penis	$T_2N_2M_0$	
66	Penis	S.	35	M	659/20	O	W.D. Sq. cell ca Penis	$T_2N_2M_0$	
67	Penis	A.S.	45	M	5167/20	B	W.D. Sq. cell ca Penis	$T_2N_3M_0$	
68	Breast	K.	50	F	1297/20	B	Pappillary duct ca. Breast	$T_2N_2M_0$	
69	Breast	A.	75	F	2245/20	B	Infilt. Duct ca.Breast	$T_2N_2M_0$	III
70	Breast	B.	70	F	2173/20	B	Infilt. Duct ca.Breast	$T_2N_2M_0$	III
71	Breast	G.	60	F	1407/20	O	Infilt. Duct ca.Breast	$T_2N_2M_0$	III
72	Breast	B.	45	F	2293/20	O	Infilt. Duct ca.Breast	$T_2N_0M_0$	II
73	Breast	R.B.	50	F	2510/20	A	Infilt. Duct ca.Breast	$T_3N_2M_0$	III
74	Breast	G.B.	70	F	2557/20	B	Infilt. Duct ca.Breast	$T_2N_2M_0$	III
75	Prostate	J.S.	65	M	124/20	O	W.D. Adenocarcinoma prostate	$T_2N_0M_0$	-
76	Prostate	M.L.	75	M	1640/20	A	W.D. Adenocarcinoma prostate	$T_2N_0M_0$	-
77	Prostate	M.L.	60	M	3006/20	B	W.D. Adenocarcinoma prostate	$T_2N_0M_0$	-
78	Colon	G.D.	40	M	1999/20	A	W.D. Adenocarcinoma sysgmoid colon	$T_2N_0M_0$	Ib
79	Rectum	S.L.	43	M	5931/20	A	W.D. Adenocarcinoma rectum	$T_1N_1M_0$	III
80	Rectum	S.B.	50	M	1676/20	O	W.D. Adenocarcinoma rectum	$T_2N_0M_0$	Ib

RESULTS OF SRCA test in so malignant tissues

The isoantigens A,B, O (H) were found to be absent in tumour cells in 78.8% cases of malignancy. The results of SRCA test in malignant tissues are shown in table – IV.

Table Iv: Results of SRCA in 80 malignant tumors

Results of SRCA	Cases	Percentage
SRCA in tumour cell		
SRCA - ve in tumour cells	63	78.8
SRCA ± in tumour cells	15	18.7
SRCA + ve in tumour cells	0	2.5
SRCA in stroma		
SRCA - ve in stroma	77	96.3
SRCA ± ve in stroma	3	3.7
SRCA in blood vessels		
SRCA + ve in bodd vessels	63	78.8
SRCA ± ve in bodd vessels	12	15.0
SRCA - ve in bodd vessels	5	6.2

Observation before and after detergent in squamous cell carcinoma of cervix – SRCA was done in 27 cases of carcinoma cervix before and after detergent. SRCA was negative (-) in tumours cells in 25 cases and patchy (±) in 2 cases before detergent. SRCA was positive (+) in blood vessels in 23 cases, patchy vessels in one case before detergent.

High power view of squamous cell carcinoma of cervix showed that SRCA was + ve in areas where tumor revealed marked anaplasia and mitosis.

Observations before and after detergent – 9 cases were studied with SRCA before and after detergent. SRCA was – ve in tumor cells in 5 cases , ± in 4 cases. After detergent it was + ve in 5 cases , ± in 2 cases and – ve 2 cases. Also after detergent there was some qualitative difference in SRCA it was strongly + ve in blood vessels and was viable in malignant cells.

Before detergent SRCA was – ve in tumor cells in 5 cases ± in one case. After detergent SRCA was + ve in Tumor cells in 4 cases, ± in one case and –ve in one case. After detergent intensity of SRCA was increased in malignant cells as well as in blood vessels.

Three cases had lymph node metastases and in all these cases SRCA was – ve.

Carcinoma oesophagus

Total 4 cases were studied. SRCA was – ve in tumor cells in 2 cases, + in 2 cases.

Carcinoma breast

Total 7 cases were studied. SRCA was – ve in tumour cells in 4 cases, + in 2 cases and + in on e cases.

Carcinoma prostate

Total 3 cases were studied . SRCA was –ve in tumor cells and + in blood vessels in all 3 cases.

Carcinomas of Large Bowel

Two cases of adenocarcinoma rectum and one case of Adenocarcinoma sygmoid colon were studied. SRCA was – ve in tumor cell in all 3 cases.

After detergent SRCA was positive in tumor tissues and also in mucous secretions.

Study of isoantigens A, B, O (H) in malignant tissues before and after treatment with detergent

Out of 80 cases of malignancies, 56 cases were studied by SRCA test before as well as after treatment with detergent.

Observations of SRCA test before and after detergent are shown in Table - V.

SRCA test was found to be negative in 45 case (80.4%) in tumour cells before treatment with detergent, while it became positive in tumor cells in 40 case (sections were from same tissues) after treatment with detergent.

Thus, we found that isoantigens A, B, O (H) which were not detected in malignant tissue, could be detected after treatment with a weak solution of detergent.

Table –V : SRCA in cancer tissues before and after detergent

S. No.	Tissue	Slide No.	Blood Group	Histopathology Report
1	Cervix	2190/20	A	P.D. Sq. Cell Ca. Cx
2	Cervix	2703/20	A	P.D. Sq. Cell Ca. Cx
3	Cervix	2702/20	B	P.D. Sq. Cell Ca. Cx
4	Cervix	4418/20	A	W.D. Sq. Cell Ca. Cx
5	Cervix	5813/20	B	Anaplastic Cell Ca. Cx
6	Cervix	2977/20	O	Undiff . Sq.Cell Ca. Cx
7	Cervix	2437/20	B	P.D. Sq. Cell Ca. Cx
8	Cervix	5765/20	AB	P.D. Sq. Cell Ca. Cx
9	Cervix	5973/20	B	P.D. Sq. Cell Ca. Cx
10	Cervix	2322/20	AB	W.D. Sq. Cell Ca. Cx
11	Cervix	2639/20	O	W.D. Sq. Cell Ca. Cx
12	Cervix	557/20	B	P.D. Sq. Cell Ca. Cx
13	Cervix	3129/20	O	W.D. Sq. Cell Ca. Cx
14	Cervix	3023/20	O	W.D. Sq. Cell Ca. Cx
15	Cervix	1456/20	B	W.D. Sq. Cell Ca. Cx

16	Cervix	2640/20	AB	W.D. Sq. Cell Ca. Cx
17	Cervix	3042/20	B	Mod Sq. Cell Ca. Cx
18	Cervix	49/20	O	W.D. Sq. Cell Ca. Cx
19	Cervix	143/20	O	W.D. Sq. Cell Ca. Cx
20	Cervix	329/20	B	W.D. Sq. Cell Ca. Cx
21	Cervix	1004/20	B	P.D. Sq. Cell Ca. Cx
22	Cervix	1263/20	A	W.D. Sq. Cell Ca. Cx
23	Cervix	528/20	AB	W.D. Sq. Cell Ca. Cx
24	Cervix	527/20	A	W.D. Sq. Cell Ca. Cx
25	Cervix	690/20	B	P.D. Sq. Cell Ca. Cx
26	Cervix	740/20	O	W.D. Sq. Cell Ca. Cx
27	Cervix	1579/20	O	W.D. Sq. Cell Ca. Cx
28	Cheek	3008/20	B	W.D. Sq. Cell Ca. Buccal mucusa
29	Cheek	1677/20	B	W.D. Sq. Cell Ca. Buccal mucusa
30	Cheek	4453/20	O	W.D. Sq. Cell Ca. Buccal mucusa
31	Cheek	2452/20	O	W.D. Sq. Cell Ca. Buccal mucusa
32	Cheek	2153/20	B	W.D. Sq. Cell Ca. Buccal mucusa
33	Cheek	5243/20	B	W.D. Sq. Cell Ca. Buccal mucusa
34	Cheek	2538/20	O	W.D. Sq. Cell Ca. Buccal mucusa
35	Cheek	2249/20	B	P.D. Sq. Cell Ca. Buccal mucusa
36	Cheek	866/20	A	W.D. Sq. Cell Ca. Buccal mucusa
37	Larynx	1959/20	B	P.D. Sq. Cell Ca. Larynx
38	Larynx	3310/20	B	W.D. Sq. Cell Ca. Larynx
39	Larynx	2223/20	B	P.D. Sq. Cell Ca. Larynx
40	Larynx	3125/20	O	W.D. Sq. Cell Ca. Larynx
41	Larynx	3997/20	B	W.D. Sq. Cell Ca. Larynx
42	Larynx	1053/20	B	W.D. Sq. Cell Ca. Larynx
43	Tongue	6229/20	B	W.D. Sq. Cell Ca. Tongue
44	Tongue	2117/20	B	W.D. Sq. Cell Ca. Tongue
45	Tongue	2120/20	A	W.D. Sq. Cell Ca. Tongue
46	Tongue	16/20	B	P.D. Sq. Cell Ca. Tongue
47	Oesophagus	2076/20	O	W.D. Sq. Cell Ca. Oesophagus
48	Oesophagus	1898/20	B	W.D. Sq. Cell Ca. Oesophagus
49	Oesophagus	5324/20	O	W.D. Sq. Cell Ca. Oesophagus
50	Penis	2079/20	B	W.D. Sq. Cell Ca. Penis
51	Penis	659/20	O	W.D. Sq. Cell Ca. Penis
52	Breast	1297/20	B	Pappilary duct Ca. Breast
53	Breast	2173/20	B	Infilt duct duct Ca. Breast
54	Breast	1407/20	O	Infilt duct duct Ca. Breast
55	Prostate	3006/20	B	W.D. adenocarinoma Prostate
56	Sygmoid colon	1999/20	A	W.D. adenocarinoma Sygmoid colon

Results of SRCA test in malignant tissues before and after detergent are shown in table – VI.

Observations in individual groups has been described earlier.

Table – VI: Result of SRCA in 56 malignant tumours before and after detergent

Results	Cases	Percentage
Before detergent		
SRCA - ve in tumour cells	45	80.4
SRCA ± in tumour cells	11	19.6
SRCA + ve in blood vessels	45	87.5
SRCA ± in blood vessels	3	5.4
SRCA - in blood vessels	4	7.1
SRCA ± in stroma	56	100.0
After detergent		
SRCA - ve in tumor cells	40	71.4
SRCA ± ve in tumor cells	12	21.4
SRCA - ve in tumor cells	4	7.2
SRCA + ve in blood vessels	52	92.8
SRCA ± ve in blood vessels	2	3.6
SRCA -ve in blood vessels	2	3.6
SRCA -ve stroma	53	94.6
SRCA ± ve stroma	3	5.4

DISCUSSION

We have studied 105 cases for the presence of isoantigens A, B, and H in normal tissues, benign lesions and malignant tumors. The specific red cell adherence test was used. We found 3 types of results of SRCA .

1. Positive reaction – i.e. presence of isoantigens A,B, AB or O (H) antigens in tissues.
2. Negative reaction – i.e. absence of isoantigens A,B, AB or O (H) in the tissues.
3. Incomplete or patchy (+) reaction – i.e. simultaneous presence of antigens A,B, AB or O (H) in some area and absence in other in the same section or even in the same field.

Our study was divided into two groups.

1) Control group

This included 18 cases of normal (non-malignant) tissues and 7 cases of benign lesions.

2) Test group

This group included 80 cases of malignancy . Out of these 56 malignant tissues were also studied after treatment with a solution of detergent .

In normal tissues SRCA test was found positive in parenchymatous tissues in all the 17 cases. The SRCA was found negative in stroma (mesenchymal tissue) i.e. fibrous tissue, adipose tissue, blood cells etc. In all the cases (observation table – I).

Davidsohn, I. and his colleagues (1972) have stated that isoantigens A,B, or O (H)were present in many of the normal tissues i.e. squamous epithelial cells of mucosa of mouth , pharynx, oesophagus, larynx, exocervix and vascular endothelial cells etc .They were absent in connective tissue, while blood cells i.e. lymphocyte , polymorph etc. basal layer of skin and exocervix etc.

Similar findings have been reported by Kovarik et al (1968).

In our study in 7 cases of benign lesions (Fibroadenoma breast and benign hyperplasia of prostate) SRCA was found positive in parenchymatous tissues and negative in stroma in all the cases (observation table – II)

Gupta et al (1972) studied 25 cases of benign lesions of breast tissue. In their study SRCA was found to be positive in epithelium of acini in all the cases.

I In test group we studied 80 case of malignant tumours SRCA was found negative (-) in tumour cells in 78.8% cases. It was patchy (+) in 18.7% cases and was positive (+) in 2.5 % cases (Observation table III).

A comparison percentage of neagtivty of SRCA in our series with other published series is shown in table – VII

Table – VII: Comparison of results of SRCA of present series with other reported series (Percentage of negativity of SRCA test

Workers	Sq. Cell carcinoma cervix	Sq. Cell carcinoma Larynx	Ca. oral cavity	Carcinoma breast	Carcinoma Prostate
Kovarik et al (1968)	85.0 %	66.0%	60.0%	-	-
Davidsohn , I. et al (1969)	95.4%	-	-	-	-
Davidsohn , I. et al (1971)	87.0%	-	-	-	-
Davidsohn , I. et al (1973)	90.2%	-	-	-	-
Debelstein et al (1974)	-	88.9%	-	-	-
Thomas et al (1976)	100.0%	-	-	-	-
Dayasi et al (1973)	-	82.8%	100.0%	-	-
Gupta et al (1973)	-	-	-	100.0%	-
Gupta et al (1973)	-	-	-	-	100.0%
Present series	88.6%	87.5%	64.7%	60.0%	100.0%

In our study, the SRCA test was found to be negative in 88.6% cases of carcinoma of cervix.

The results were almost similar to the results obtained by other workers viz. Kovarik et al (1968) : Davidsohn , I.et al (1969, 1971, 1973) (Table –VII).

Also in case of carcinoma larynx and prostate our results almost tally with the results of other reported serried 9 table – VII)

In 80 cases of malignancy, 50 cases had metastases. In this group SRCA was negative in 92 % cases, it was patchy in 6 % and positive in 2 % cases (observation table – III).

In a study by Davidsohn, I. (1972) no antigen could be detected in 92 % cases with metstases. Partial (±) loss was present in 4% and there was no demonstrable loss of antigens in 4% cases. He concluded that –

1. Loss of A,B and H antigens in a primary carcinoma can be interpreted as indicative of possible or even already present metastases. So a negative test in a primary carcinoma should encourage intensive search for metastases.
2. A positive adherence test in a primary carcinoma is an indicator of presence of antigen and speaks against the presence of metastases.

We also came to the same conclusions. Thus, in a case of primary carcinoma the SRCA test is valuable diagnostically in the early lesion and prognostically in the advanced disease.

The presence of isoantigens in normal tissue brings up the question of origin of these antigens. Are they produced by the cells in which they are found?. Consequently the failure to demonstrate these antigens after a carcinoma has

developed in the same tissues in which they are present normally may be due to their loss or to the failure to store them.

No exact answer to this question is available a present time. Kovarik et al (1968) suggested that the failure to demonstrate blood group isoantigens in cancer might be due to the following factors.

1) Inability to produce blood isoantigens.
2) Inability to store.
3) Destruction of isoantigens by cancerous transformation.
4) Masking of isoantigens.

Although several authors confirmed the observations of non – demonstrability of blood group isoantigens , yet none of these authors theorised or suggested any possible mechanism .

It has also been shown that serum contains blocking factors which under appropriate situations may inhibit the cytotoxic effects of lymphocytes. There has been several studies to find out the role of blocking factors interfering with immunological rejection of tumors. Hellstrom and hellstrom (1966) ; Joshi and Sheshdhari (1974) : Beldwin and Robins (1976) had shown that blocking factors might be of the nature of specific immune complexes consisting of tumor antigens and tumor specific antibodies and might inhibit the cell mediated immunity in tumor bearing host by inducing a blocking reaction in tumor cell surface. It was, therefore, presumed that these blocking factors might be coating the cell surface.

So it may be considered reasonable that the determinants of blood group isoantigens on the tumor cell surface may get masked or obscured by blocking factors.

We also studied 56 case of malignant tumours after treating them with 1% solution of detergent. In this group SRCA was found negative in 80.4% cases, patchy in 19.6% cases in tumor cells before detergent. After treatment with detergent SRCA was found positive in 71.4% case and patchy in 21.4% cases in tumor cells (table-V) .So we presume that the blocking factors were washed away by treatment with solution of detergent. The mechanism of this being and unexplored field invites future studies.

Summary and Conclusion

Total 105 cases were studied for the presence of isoantigens A, B and 0 (H) in normal tissues, benign lesions and malignant tumours by specific red cell adherence (SRCA) test.

The SRCA test was positive (+) (indicating presence of isoantigens in all the normal tissues and benign lesion.

In malignant tissues SRCA test was negative (indicating complete loss of isoantigens) in 78.8% cases. While the test was patchy (±) indicating partial loss of isoantigens in 18.7% case. Isoantigens were demoanstrable only in 2.5% cases of malignancy.

Out of 80 cases of malignancies, 50 cases had metastasis. In this group SRCA test was negative (loss of isoantigens in 92% cases.

Fifty-six case of malignancies were also studied after treating the paraffin sections with a weak solution of detergent. In this group similar sections from the same tissues were studied simultaneously after treating them with detergent as well as without detergent. The SRCA test was negative in tumour tissues (loss of isoantigens) in 80.4% cases, while it was patchy (±)(partial loss) in 19.6% cases before detergent. After detergent it became positive in tumour tissues (presence of isoantigens) in 74.4% cases while it was patchy (partial loss of antigens) in 21.4% cases.

We came to the following conclusions.

(1) In borderline cases when pathologists disagree in their interpretation of benign or malignant nature of a primary neoplasm of an organ or tissue that normally contains an isoantigens or isoantigens of the ABO group a negative specific red cell adherence test indicates the loss of isoantigens and favours the diagnosis of carcinoma; while a positive reaction indicates the presence of isoantigens and makes the diagnosis of carcinoma less likely.

(2) A negative reaction (loss of isoantigens) in primary carcinoma suggests the possibility or probability or even presence of metastases, while a positive reaction (presence of isoantigens) in primary carcinoma makes the presence of metastasis unlikely.

(3) The mechanism of failure of demonstration of isoantigens ABO in malignant tissues can be explained possibly by masking of isoantigens; by some blocking factors, because in our study we found that detergent treated malignant tissues revealed a strongly positive SRCA test (indicating presence of isoantigens) which were originally consistently negative for blood group isoantigens in the same untreated sections of malignant tumors .the blocking factors were possibly removed by the action of detergent on cell surface thus unmasking he isoantigens.

(4) The adherence test may be useful in the early diagnosis and prognosis of carcinomas of organs and tissues that normally contain the three isoantigens.

(5) SRCA is considered as an excellent test for retrospective studies as the age of paraffin embeded tissues did not affect the sensitivity and specificity of the test (this was established during standardisation procedure before undertaking the work).

(6) SRCA though could not be proved superior to histopathological examination, requires to be studied further. It is possible that with more experience and cultivation of observatory acumen on quantitative adherence of erythrocytes, one may be able to spot a precancerous state earlier than histological changes.

Bibliography

1. Aikat , B.K., Indian j. Patho. and Bact. 17:2, 1974
2. Aird, I., Lee, R.D., Roberts, J.A.F. , A,B,O blood groups and cancer of oesophagus , and pituitary adenoma. Brit. M.J. 1:1163:1166,1960
3. Allen, P.Z., Immunochemical studies on blood groups, J. immunol . 82:358,1959.
4. Barber , M. Dunsford, I., Excess of blood group substance A in the serum of patients dying with carcinoma of stomach Brit m.J. I; 607,1959
5. Bhatia, H.M., Techniques in blood group serology, Indian council of Medical Research Technical Report series No. 13,1972

6. Cohen, F., Zuelzer , W., Evans,M, Identification of blood group antigens and minor cells population by fluorescent antibody method. Blood , 15:884.1960
7. Coo mbs , R.r.A, D. Rouillard I.M., Bedford, A and B group antigens in human epidermal Cells demonstrated by missed cell agglutination, Lancet. 1:461,1956.
8. Cyril, J., Immunological analysis of inner and outer, membrane of Escherichia coli, by crossed immnoelectrophorsis. J. Bact. 133.307,1978
9. Dablsteen, E., Quantitative determination of blood group substance A of oral epithelial cells by immunofluorescence, and immunoperoxidase methods., Acta tath. Microbilo. 80:847,1972
10. Dablelsteen, E., Loss of blood group substance A in oral carcinoma, Acta Path. Microbiol. 1973
11. Dablelsteen , E.Blood group substances in carcinoma of the larynx., Acta otolaryngologica. 77:360,1974
12. Davidsohn, I., Kovarik, S., Lee, C.L., A,B,O substances in gstrointestial carcinoma, Arch. Of pathlol. 81:381,1966
13. Davidsohn, I., Kovarik , S., Ni , L.Y., Isoantigens A,B, and H in benign adn malignant lesions of cervix Arch of athol . 87;306-314,1969
14. Davidsohn, I., Ni,L.Y, Immunocytology of cancer. Acta cytology (Batimore), 14:276-282,1970
15. Davidsohn, I., tissue isoantigens A,B and h in carcinoma of stomach. Arch of athol. 92:456,1971
16. Davidsohn, I., Early immunological diagnosis and prognosis of Carcinoma, Am. J.clin. Patho. 57:715-730,1972
17. Davidsohn, I., The effect of squamous cell carcinoma Antigens A,B, and H . Arch of patho. 95:132,1973
18. Glynn, L.E., Holborow ,E.J. Johnson G.D. The distribution of blood group substances in human gastric and duodenal mucosa. The lancet . 2:1083-1088,1957
19. Gynn, L.E. Holborow, E.J. Distribution of blood group substances in human tissue Bri t. M. Bulletin . 15:150-153,1959
20. Gupta ,R.K. , Shchuster, R, Isoantigens A,B, and H in benign adn malignant lesions of breast Am. J. of patho. 72;253-260,1973
21. Gupta ,R.K. , Shchuster, R, Christian , W.D., Loss of isoantigens A,B, and h in prostatic carcinoma Am J. of patho. 70:439,1973
22. Hakamori, S., Investigations on cellular blood group substance Isolation and chemical composition of blood group substances A,B and H, Quoted by wintrobe (1974)
23. Hartman, G., Group antigen in human organs: selected Contribution to the literature of blood groups and Immunology. Cited by Davidsohn, I .(1972)28
24. Kabat, E.A., Immunochemical studies on blood group Biochem. 4;1632,1965
25. Kay, H.E.M., A and B antigens in normal and malignant cells. Brit. J. Cancer.11:409,1957
26. Kovarik S., Davidsohn. I., Stefskal, R., A,B,o antigens in cancer Detection with mixed cell agglutination reaction. Arch patho. 86:12-21,1968
27. Nairn, R.C., Loss of gastrointestinal specific antigen in neoplasia. Brit. M.J. 1;2:1791,1962
28. Szulman , A.E. The histological distribution of blood group substances A and b in man. J. exp. Med. 111:785,1960.
29. Szulman, A.E., The histological distribution of blood group substances In man as discussed by immunfluorescence. II. The H antigen and its relation to A and b antigens. j.exp med. 115:977,1962
30. Weiner, A.S.: Observation on the serological specificity of the ABO blood group system. Brit. J.Haemat. 24;195,1973

ABBREVIATIONS

BPH	:	Benign prostatic hyperplasia
Ca.	:	Carcinoma
Cx.	:	Cervix
F.	:	Female
Infilt.	:	Infiltrating
Inv.	:	Invasive
M.	:	Male
MCAR	:	Mixed Cell Agglutination Reaction
M.D.	:	Moderately differentiated.
No.	:	Number
Parenchym	:	Parenchymatous
P.D.	:	Poorly differentiated
S.No.	:	Serial number
Sp.	:	Specific
Sq.	:	Squamous
SRCA	:	Specific Red Cell Adherence.
Syg.	:	Sygmoid
T.N.M.	:	Tumor Node Metastasis
Undiff	:	Undifferentiated
Yrs.	:	Years.
W.D.	:	Well differentiated
+ve	:	Positive
-ve	:	Negative
±	:	Patchy