

Original Research Article

Study of serum fucose level in breast malignancy

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ABSTRACT

Background: Although for advanced tumour of breast have certain obvious physical characteristics, seldom is such physical finding seen in early malignant breast tumour. Keeping all these in mind, the present study aims at estimation of serum fucose which may help in early detection of breast malignancy.

Methods: The present study was carried out over a period of one and a half year. A total of 68 patients were studied. Out of these 68 cases, 32 cases were having breast malignancy. After noting details history, thorough examination was done according to the proforma. Investigations were carried out as shown in proforma. Estimation of serum fucose was done in all these cases. Estimation of serum fucose was also done pre-operatively and in post-operative period.

Results: It is evident from this that 70% of malignant lumps were seen in upper and outer quadrant of breast, 25% lumps in lower quadrant. It is obvious that as age advances average serum fucose level in breast cancer, starts decreasing. Thus younger patients have high Serum level as compared to old patients. Rise in serum level with the progression of stage of breast cancer. There was significant reduction observed in serum fucose level after surgery ($p < 0.0001$).

Conclusions: The estimation of serum fucose level can be used to screen cases of breast malignancy though neither as a strong arbiter, nor as a distinct diagnostic test. Its use is only as an ancillary investigation which may provide collaborative evidence only.

Keywords: Serum fucose level, Breast malignancy, Diagnostic test, Surgery

INTRODUCTION

It was Sheltzer and his associates who first demonstrated the association between growth of human tumors and elevation of serum glycoproteins.¹ Serum fucose estimation in breast- tumors were done by Macbeth and Macbride. In their first study, an elevation of plasma non-seromuroid protein bound fucose was found in patients with carcinoma of Breast even in the absence of wide spread metastasis. In their second study, they have not found any significant difference between serum fucose of patients with carcinoma breast and patients with benign breast mass.²

Glycoproteins comprise of a large number of substances of diverse biological importance and behavior, namely: antibodies, enzymes, hormones, blood group substances, mucus secretions, membranes, and collagens. The levels of glycoproteins are elevated in a number of diseases including cancer.³

Glycoproteins contain galactose, mannose, glucosamine, galactosamine, sialic acid, or fucose as the carbohydrate residue.⁴ It has been reported that tumor cells modulate their surface by increasing fucosylation levels (addition of 1-fucose at the terminal end of the oligosaccharide chain) to escape recognition, which contribute to several abnormal characteristics of tumor cells, such as decreased

adhesion and uncontrolled tumor growth.⁵ Hence, monitoring serum or tissue fucose levels could be a promising approach for the early detection, diagnosis, and prognosis of various cancer types.^{4,5}

At present there is obvious need for specific information regarding nature of breast-mass impressions of character of breast mass are based on physical examination and more recently on mammography. Although for advanced tumour of breast have certain obvious physical characteristics, seldom is such physical finding seen in early malignant breast tumour.

Keeping all these in mind, this study aims at estimation of serum fucose which may help in early detection of breast malignancy. Objective of the study was to determine serum fucose level in various breast condition.

METHODS

The present study was carried out at Dhanalakshmi Srinivasan Medical College and Hospital, Siruvachur, from January 2018 to June 2019. A total of 68 patients were studied. Out of these 68 cases, 32 cases were having breast malignancy, 6 cases had benign lesions of breast, 15 cases had acute breast abscesses, and 15 were control individuals.

Out of the 15 patients, 8 were healthy. Female blood donors and remaining 7 male young healthy blood donors, all these had donated blood in our blood bank. In this study, we also include eight cases which were having malignancy of other organs i.e. carcinoma penis, carcinoma larynx etc.

Procedure

After noting details history, through examination was done according to the proforma. Estimation of serum fucose was done in all cases. Estimation of serum fucose was also done Pre-operatively and in post-operative period i.e. on 3rd and 7th post-operative day. In the cases of breast abscess on the very next day i.e., after 24 hours, after the abscess, is drained blood is withdrawn and serum fucose was estimated.

Method of estimation

In the present study, adopted the technique of Dische and Shettles with Klinzeler's modification.⁶ In this method, sample of about 5 ml of venous blood is withdrawn on a fasting state in a dry syringe. It was observed that dry syringe was essential for collection to avoid early haemolysis of R.B.C.

In this study estimation of serum fucose was done within an hour after collection of sample. Dische and Shettle's method includes following reagents including 95% ethanol, 0.1 N NaOH, 3% cysteine hydrochloride,

standard solution of l- fucose, concentrated sulphuric acid.

Apart from present method, there was one more method suggested by Goinell and Bardwill. It is called biuret reaction or biuret technique. It meant the expression of final result as mg fucose/mg of serum protein. Thus Gornell and Bardwill were able to minimize the effect of serum protein – variation.

Other methods of estimation of serum fucose level include Gorky and Houck uses trichloroacetic acid instead of 95% Ethanol for preparation of proteins. Another sensitive method is suggested by Murakami and Klinzler which consists of reduction of fucose to fucitol with the help of sodium or dehydride labeled with tritium. This is followed by separation on thin layer chromatography and counting the radioactivity associated fucitol. Literature also mentioned about determination of tumor fucose level, however it is not included in the present study.

Collection of sample

About 5 ml of venous blood was withdrawn from patient in fasting state in dry syringe. Blood was collected in plain sterile bulb and was placed in hot water bath immediately.

Separation of serum

For this purpose, sample was kept in hot water bath for 1 hour. After that supernatant serum was taken in plain sterile test tube and centrifuged for 10 minute 3000 r.p.m. again the supernatant fluid was taken and centrifuged till clear yellow serum is obtained. Based on the method of Dische and Shetties calculation of serum bound fucose is a simple determination.⁶

A venous blood sample was obtained from a patient in fasting state, allowed to clot and the cells separated by centrifugation one millimeter of 95% ethyl alcohol was added to 0.1 ml of serum in order to effect protein precipitation. The sample was centrifuged and the precipitate was washed and re-suspended in 1 ml of ethyl alcohol.

Centrifugation and washing are carried out again and the precipitate was re-suspended in 3 ml of 0.1 N normal sodium hydroxide to resolubilize the proteins. One ml of alkaline suspension was placed in a test tube to which is added 4.5 ml of concentrated sulphuric acid and the mixture was heated in boiling water bath for 3 minutes.

A colour product was formed in this reaction. Duplicate samples are run and to one of the samples is added 0.1 ml of 3% cysteine hydrochloride. Cysteine is not added to other samples in order to allow for correction of colours developed by the reagents.

The colour product formed by Fucose has maximum absorption at 396 mu and at most no absorption at 430 mu. Other sugars which also form a colour product with cysteine and absorb maximally at 396 mu, have an equal absorption at 430 mu also. So that the difference in absorption between these two wave lengths is the absorption due to fucose alone. A standard consisting of 20 mg fucose per ml and water blank is processed with each set of samples.

Statistical analysis

Descriptive statistics such as mean, SD and percentage was used to present data. Comparison of serum fucose level between pre and post operative cases was performed by using paired t-test. A p-value less than 0.05 were considered as significant.

RESULTS

In present study, it is observed that majority of the patients belongs to 41-50 years (53.1%) followed by 51-60 years (28.1%), 31-40 years and 61-70 years (9.4%) each. Out of 40 cases of breast lumps studied, 32 patients (80%) had malignant tumours, 6 patients (15%) had benign tumour, 1 patient (2.5%) was having cystosarcoma phylloid, and 1 patient (2.5%) of sarcoma breast. Out of 32 cases of malignant lump one patient was male (carcinoma of male breast).

Table 1: Distribution of cases.

	Benign	Malignant
Upper inner	2	1
Upper outer	-	28
Outer lower	2	2
Inner lower	-	1
Central	1	-

The serum fucose levels in 15 control cases (both males and females) ranged from 0.25 mg to 11.6 mg%. The average serum fucose level being 3.92 mg%. In 10 cases of benign lumps cases the average was 7.8 mg% and in 32 cases of malignant lump the average serum fucose level was 25.85 mg%.

The difference between serum fucose levels of benign, control and malignant were subjected to "T" test for statistical analysis and it was found that the rise in serum fucose level in level from normal control to benign cases was significant, while rise in level from benign to malignant group was highly significant. The rise in level

Table 5: Changes in serum fucose level in pre and post-operative.

	Pre	Post	Mean difference	T-value	P-value	Remarks
Serum fucose level	25.6±11.7	13.2±6.7	12.4	8.53	<0.0001	Significant

from normal to malignant group was also. All the findings are summarized in the adjacent tables.

Table 2: Relationship of age group with serum fucose level (only carcinoma).

Age (years)	Number	Average serum fucose level (mg%)
31-40	3	32.4
41-50	17	28.5
51-60	7	28.2
61-70	5	25

Out of 32 cases of malignant lumps 30 patients (93.8%) had serum level above 25.5 mg% i.e., above the average level of benign cases 7.8 mg%. It was also found that serum fucose level tends to remain on higher side in breast cancer in the younger age group than in older patients. It is obvious from this table that as age advances average serum fucose level in breast cancer, starts decreasing. Thus younger patients have high serum level as compared to old patients (Table 2).

It was observed that, rise in serum level with the progression of stage of breast cancer (Table 3).

Table 3: Rise in serum fucose level with progression of stages in cancer breast (according to Manchester's classification).

Stage	Number	Average serum fucose level (mg%)
I	4	16.8
II	15	25.6
III	15	27.2
IV	4	35.4

Table 4: Distribution of breast lumps and serum fucose level.

Breast lumps	Number	Average serum fucose level (mg%)
Normal	15	3.92
Benign	18	7.8
Malignant	32	25.65

This table shows serum fucose levels in normal and cases of breast lumps. It is evident that serum levels in malignant cases the above normal level in all the cases (100%). In benign cases these are just above normal 70% (Table 4).

There was significant reduction observed in serum fucose level after surgery ($p < 0.0001$) (Table 5).

DISCUSSION

Carcinoma breast is a commonest female cancer in India, after carcinoma of oral cavity and cervix. Despite recent advances in treatment by surgery, radiotherapy etc., the mortality from breast cancer has not changed over last 30 years^{7,8}.

The main factor responsible for bad prognosis and poor results after treatment is delay in diagnosis. If some method was available for early diagnosis of breast cancer, a major break-through in the results of treatment would have come.

For the first time, Sheltzer and his associates observed certain relationship between growth of human tumour and rise in Serum glycoproteins¹. It was Macbeth and Bekesi, who positively showed that it is only protein bound fucose which is always raised in localized breast carcinoma². But amongst them nobody knew the exact reason for this elevation.

It has been postulated that it could be due to increased production by liver due to stimulation by the existing tumour or due to the synthesis of glycoprotein by the neoplastu tissue itself. In the present study, we have estimated serum fucose in cases of breast lesions which included breast abscesses, benign lesions of breast and carcinoma of breast.

Age incidence

Rosato and Francis observed that breast malignancy was common in 6th And 7th decade of life.⁹ Similar findings were also observed by Arya and Bhatnager, majority of patients belongs to 41-50 years (43.2%).¹⁰ In present study, it is observed that majority of the patients belongs to 41-50 years (53.1%) followed by 51-60 years (28.1%), 31-40 years and 61-70 years (9.4%) each.

Quadrant affected

In cases of carcinoma breast we have analyzed the data in relation to the quadrant of affected breast. It may be upper outer, or lower inner etc. In the present study out of 32 patients with malignancy, 28 patients had lumps in their upper and outer quadrant and in two patients the lower and outer quadrant. One patients had upper and inner quadrant involvement and the last one had lump in lower and inner quadrant.

We have examined and investigated 68 patients with breast- mass. Out of these 38 patients (80%) had malignant tumors, 6 patients were having benign lesions (15%), 1 patients had cystosareoma phylloids (2.5%) And 1 patient was having careinoma male- breast.

The present study also includes 15 patients in control group which are regular healthy blood donors and 15 cases of acute breast abscesses. The presence of fucose as a constituent of glycoprotein was identified by Dische and Osnos. The serum fucose level was assessed by them in 20 normal individual was 8.9 ± 0.6 mg%.

In India the serum fucose level was further determined in 40 normal Indian Children by Sharma and Sur. In their study, the average normal serum fucose was $4.6 \text{ mg} \pm 0.13 \text{ mg}\%$.¹¹

Compared to the level obtained in Dische and Shettles' series, these figures (Indian study) are quite low.

Sharma and attribute these low figures to the fact that our Indian food comprises mainly of carbohydrates and low in proteins as compared to high protein diet of west.¹¹

Rosato and Francis also studied the problem of serum fucose level in breast malignancy in detail.⁹ He studied series of 15 patients (patients with histologically proved malignancy). The average level for malignancy was 13.3 mg%. His study also included 46 patients with histologically proved benign mass.

In their study, average serum fucose level was found to be 8.6 mg %. It was only in 2 patients was malignancy who showed level below 12 mg %. Conversely 13 percent of the patients with benign masses showed serum fucose level 12 mg% or greater. It would seem from these results that in a given patient with breast- mass, the finding of serum fucose level 12 mg % shows suspicion of malignancy. Conversely the level below 12 mg% is more suggestive of benign lesion.

In Arya and Bhatnager study reported, out of these 51 patients with breast lump 39 patients (76.47%) had malignant tumor, 10 patients (19.61%) had benign tumor, 2 cases stayed in Hospital, labeled as carcinoma left hospital without Surgery.¹⁰

Normal serum fucose ranged from 4 mg % to 7.2 mg %, average level being 4.92 mg %. In the benign lesions of breast the average was found to be 6.15 mg %. Out of 41 cases of malignancy of breast from their series, 37 patient (90.2%) had serum fucose level above 6.15 mg % which is the average level for benign lesion and also of control patients. The serum fucose level remains on higher side in young patients rather than seen in older patients.

Rosato and Francis, Sheltzer et al studied this problem in its depth, out of these 150 patients, 46 patients were the case of carcinoma (histopathological proved).

The normal average fucose level was found to be 3.35 mg%. These 46 patients showed elevated levels. This was all regarding the various 1 series of observations and results in past.

In the present study, amongst healthy 15, 8 are female one and 7 are male ones. The serum fucose in healthy group ranged from 0.25 mg% to 11.4 mg% the average level being 3.92 mg%.^{1,9} This level is definitely lower than the level estimated in Dische and Shetties series (8.9 mg%).

It is also low as compared to one which is estimated by Sharma and Sur in their series (4.6 mg). And to one from Arya and Bhatnager's series (4.92 mg %).¹⁰ The exact reason for the lower values, is not known. It may be due to the fact Indian food comprises mainly of carbohydrates and low in proteins.¹¹

Our data regarding the cancer breast is definitely a conclusive one. The serum fucose level in these 32 patients of cancer breast ranged from 6.0 mg to 60.0 mg% Average level being 25.65 mg%. This average level is higher than was seen in Arya and Bhatnagar Series i.e. 8.09 mg%.¹⁰

Our average level is also on higher side as compared to Rosato study which included 46 patients and the serum fucose was 8.6 mg%.⁹ Regarding the benign lesions of breast, this series includes only six cases. In them serum fucose level ranged from 2.4 mg to 19 mg%. The average level is 7.8 mg%. This level is higher when it is compared to Arya and Bhatnager Series (6.15 mg%).¹⁰

This study also includes 15 patients of acute breast abscesses. Average Serum Fucose level in these cases was 4.5 mg% which was above normal control level. Serum fucose level was again estimated after 48 hrs. This post-operative reading showed definite decline of the serum fucose to near normal control level. Our readings collaborate with the readings obtained by Arya and Bhatnagar in their cases of breast-abscesses.¹⁰

In the present study, there was significant reduction observed in serum fucose level after surgery ($p < 0.0001$). A study reported a significant increase in serum protein-bound fucose in patients with advanced cancer and other diseases. In their study of 15 cases of advanced cancer they found the serum fucose levels to be 14.2 ± 2.1 mg% in comparison to the normal level of 8.9 ± 0.6 mg%.¹²

CONCLUSION

There is significant rise of serum fucose level in malignant breast lumps and this rise in the level is directly proportional to the stage of Breast Cancer and inversely proportional to the ages of the patients. The serum fucose level tends to decline gradually after the removal of tumour mass, more in cases without

metastasis rather than those with metastasis. The estimation of serum fucose level can be used to screen cases of breast malignancy though neither as a strong arbiter, nor as a distinct diagnostic test. Its use is only as an ancillary investigation which may provide collaborative evidence only.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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