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Estimation of Prolactin Level in Women with Carcinoma Breast

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Introduction

In recent times the incidence of tumors of breast is quite alarming all over the world. In 2012, nearly 1.7 million new cases of breast cancer is reported worldwide.

The occurrence of breast tumors is considered to be due to very many risk factors. Based on epidemiological data, risk factors for breast cancer fall in 3 main groups: Genetic, endocrine and environmental. Each may be of major, intermediate or minor importance. Major risk factors include 1. Female gender 2. Increasing age 3. History of previous breast cancer 4. Family history 5. Genetic factors (mutation of BRCA 1 or BRLA 2), appear to account for over half of all the identifiable breast cancer families.(1) Intermediate risk factors include alcohol, diet, nulliparity, early menarche, late menopause, oral contraceptives, hormone replacement therapies, benign proliferative breast disease, irradiation etc.

The fact that Prolactin (PRL) is important in all phases of breast development and critical to breast control has been established by careful studies.(2) Development of breast involves coordinated action of many hormones including prolactin, estrogen, progesterone, gluocorticoids, insulin, growth hormone and thyroid hormone. Duct growth is promoted by estrogen, alveolar development is controlled by Prolactin & progesterone, and lactation by Prolactin.(3)

The prolactin level is found to increase during menarche as well as in the premenopausal women.(4) Higher levels are also found during pregnancy both in the mother and the fetus. At term prolactin level is 10 times as non pregnant women. High levels of prolactin appear to be essential for the initiation of lactation. Reduction in the prolactin level following menopause has been reported.(5) Peak levels of prolactin occur during middle to end of night and lowest at midday.(6)

The role of prolactin in galactorrhea and infertility in women is largely known. Locally produced prolactin acts as an autocrine/ paracine factor in breast cancer evolution.(7) Though little is produced in breast, due to local availability it plays an important role in tumor formation.

JAK2 is required for prolactin mediated maintenance of differentiated alveolar cells. Prolactin may promote breast cancer via JAK 2/STAT 2 signaling pathway.(8) Breast tumors also express higher levels of prolactin receptor when compared to healthy tissue. Conflicting reports are found regarding the involvement of prolactin in cases of carcinoma breast. Hence this study is taken up to find whether the increasing incidence of breast cancer has any relation to the prolactin level of the individual and also its relevance.

Materials and Method

Type of study: Cross sectional study
Study design: Randomized control trial
Study setting: The Study was done at Government Rajaji Hospital attached to Madurai Medical College, Madurai after obtaining Institutional Ethical Clearance.
Study population: A total of 39 female patients between the age group of 35-65 years have been studied over a period of one year. 27 of them had malignant tumor (invasive ductal carcinoma) of the breast. Patients were divided into these groups following histopathological examination of the tissue removed at surgery. Of them 12 were premenopausal and 15 were postmenopausal. Twelve normal women having similar age, weight and menstrual history were selected and served as control. Of them 7 were premenopausal and 5 were postmenopausal.

After obtaining informed consent from the selected individuals, the proforma was filled in eliciting the required data to find the possible risk factors. The subjects were divided according to their menstrual status with reference to their case history. Subjects were classified as postmenopausal if two years had elapsed since their last menses. In premenopausal subjects blood samples were taken during the follicular phase of the menstrual based on their case history. Pregnant /lactating women were excluded from the study.

All patients included in this study were without any prior treatment. As far as could be ascertained, no patient was receiving phenothiazines, L-Dopa, monoamine oxidase inhibitors, or other drugs known to affect the secretion of Prolactin. Each patient had a blood sample taken between 11.00 am and 5.00 pm and blood was allowed to clot and the resulting serum was frozen at -20˚ C until assayed. Serum Prolactin level was estimated by Immuno Radio Metric Assay (IRMA).
Principle of the test: This immunoradiometric assay is a non competitive assay technique to quantitate

prolactin in serum sample. In IRMA, two antibodies generated against different portions (epitopes) of the same antigen are used. The capture antibody is coupled to magnetic particles and used as the solid phase and the detection antibody is radiolabelled with ^{125} I. When antigens (standards or sample) are incubated with the tracer and the solid phase, it simultaneously binds to both the antibodies in a bridge or sandwich fashion. Their entire complex remains bound to the magnetic particle. The bound radioactivity associated is then quantitated using a gamma counter calibrated for ^{125} I. The concentration of prolactin in the sample is directly proportional to the radio activity. The concentration of prolactin of unknown sample is read off by the interpolation from the curve.

Summary of the assay producer: Sample or Standard is assayed in duplicate.

Step 1- Addition: To the assay tubes added successively are 1) 50 micro liters of standard or sample 2)100 micro liters of tracer 3)100 micro liters of solid phase with constant stirring mixed well.

Step 2: Incubation: Overnight without stirring at 25˚C

Step 3: Counting: 2ml of wash buffer added to each tube except total tube (contains 100 micro liters of only Tracer) .Washing repeated twice. The centrifugate is counted in gamma counter for one minute. Graph plotted.

Calculation of results

1. Subtract the back-ground from all counts to get corrected counts.
2. Take average of all duplicates.
3. Average counts of tubes 1 & 2 are called total counts.
4. % Bound (B) / total (T) = Corrected average counts of the standards / Corrected average counts of the total tubes.
5. Determine sample values from the standard curve.

Statistical Analysis: The quantitative data was checked for normality and summarized using mean/median and standard deviation/ interquartile range as appropriate. Categorical data is summarized as frequencies and percentages. Prolactin level in carcinoma breast group and control group was compared using student t test & chi square test. An arbitrary cut off of 0.05 was used to interpret significance of p value.

Results

Table 1: Association of increased prolactin level with carcinoma breast

Chi-square test:

	No of subjects	Increased Prolactin Level	Normal Prolactin Level
Carcinoma Breast	27	19	8
Control	12	0	12

$X^2 = 13.77$ P = 0.0002 P < 0.05 significant

Table 2: Association of increased prolactin level with carcinoma breast

Student t Test:

Prolactin level	t	p
Carcinoma X Control	3.98	0.00037*

P < 0.05 significant

Table 3: Comparison of prolactin level between pre and postmenopausal control group

Group	Number of Subjects	Mean Prolactin level ng/ml	Standard Deviation
Control	12	7.51	3.90
Premenopausal	7	9.44	3.43
Postmenopausal	5	4.79	2.88

Table 4: Comparison of prolactin level between pre and post menopausal Carcinoma breast group

Group	Number of Subjects	Mean Prolactin level ng/ml	Standard Deviation
Carcinoma Breast	27	52.40	38.696
Premenopausal	12	74.76	44.83
Postmenopausal	15	34.36	20.36

Table 5: Comparison of prolactin levels between Early and Advanced carcinoma breast group

Carcinoma Group	Number of Subjects	Mean Prolactin level ng/ml	Standard Deviation
Early Disease Status	11	42.997	37.37
Advanced Disease Status	16	58.80	39.33

Early disease status includes stages I & II
Advanced disease status includes Stages III & IV

Table 6: Comparison of Prolactin level between 1. Pre & Post menopausal women 2. Early & Advanced disease status

Prolactin level	t	p
Premenopausal X Postmenopausal	3.12	0.0044*
Early X Advanced Disease status Disease status	1.05	0.3051
Early Disease Status Premenopausal X Postmenopausal	3.46	0.0071*
Advanced disease Status Premenopausal X Postmenopausal	1.77	0.0984

P < 0.05 significant

Observation:
Among 12 controls all were with normal prolactin level < 20ng/ml
Among 27 carcinoma breast cases, 70.4% were with increased prolactin level
29.6% were with normal prolactin level.
Considering the incidence of risk factors in the patients with carcinoma breast the findings in the present study were as follows:
14 – 51.9% - were with no known risk factor
4 – 14.8% - were with family history of tumor breast.
5 – 18.5% - were with nulliparity
3 – 11.1% - were with Age at first pregnancy more than 30 years.
6 – 22.2% - were with no breast feeding done.

Discussion

Evidence that Prolactin was a carcinogen in rat mammary cancer was presented in 1969.[9] Since then there are numerous reports in the literature of investigation of relationship between prolactin and various aspects of breast cancer. The majority of these have concentrated on a possible etiological association between the incidence of breast cancer and abnormality in secretion. Many have found suggestive but by no means definitive evidence of such an association. Studies for prolactin level in cancer breast patients have also produced conflicting reports.

Thus elevated prolactin levels are reported in certain groups of cancer breast patients[10] and in high risk families.[11] Despite these observations several reports suggest that prolactin levels are normal in women with breast cancer.[12,13,14]

The present study has demonstrated that there is a significant increase in the level of prolactin in cancer breast patients when compared with control group. p value is less than 0.05 as shown in Table 1 & 2. Mean serum prolactin level in the control group is 7.51ng/ml (Table 3) which is well below the normal value of 20ng/ml and is definitely less when compared with the carcinoma group where the mean value observed is 52.40 ng/ml (Table 4).

Also the level of prolactin is significantly higher in premenopausal group (74.76ng/ml) when compared with postmenopausal group (34.36ng/ml) in carcinoma breast patients as shown in Table 4&6. These findings are in agreement with the study done in 1986 on plasma lipids and prolactin in patients with breast cancer[15] where high levels of prolactin (>100 ng/ml) were found more in premenopausal invasive breast cancer patients with distant metastases than in postmenopausal group. There is no significant difference in prolactin level between early and advanced carcinoma group (Table 6) even though a higher mean prolactin level was found in advanced (58.80ng/ml) than in early carcinoma group(42.99 ng/ml) as shown in Table 5.

Unopposed estrogen rendered the breast more susceptible to carcinogenesis.[16] Estrogen stimulates prolactin secretion and also up regulates human prolactin receptor gene expression in the progress of the disease.[17]

Feeding high fat diets causes elevation of plasma prolcatin concentration in man.[18] Increasing parity was related to a steady decrease in prolactin concentration.[19] Thus bodyweight and parity might influence breast cancer risk by being associated with changes in blood prolactin concentration. It was also evident that suppression of high prolactin levels increased the response to cytotoxic therapy in tumor patients.[20]

The interpretation of the result obtained in present study depends on the confidence with which a single prolactin estimate between 9.30 a.m to 5.00 p.m represents the prolactin status of the patient. The defined time period was chosen to avoid major variation due to the diurnal rhythm of prolactin. By 9.30 a.m the fall of prolactin levels from their nocturnal sleep induced peak has reached a plateau.[21]

Though stress increases the prolactin level, a number of investigations have concluded that venipuncture rarely induces prolactin release.[22] Studies have shown that prolactin levels vary depending upon the phase of menstrual cycle.[23] In the present study since blood samples were taken only in the follicular phase of the premenopausal subjects, the difference found in Prolactin level cannot be attributed to the phase of menstrual cycle.

Similarly because the blood sample is taken prior to any treatment, the difference in the level cannot be attributed to surgery induced increased prolactin level or chemotherapy or Hormonal therapy induced suppression of prolactin level. Also studies show that routine breast examination does not alter serum prolactin level.[24] Though artefactual high prolactin levels may occur due to pulsatile release, number of investigations have concluded that frequent sampling does not improve the value of a result.[25] These studies suggest that the result of the single estimate of prolactin level in the present study may be viewed with confidence.

Although prolactin has been long suspected to be involved in the progression of human breast cancer, the failure of clinical improvement by treatment with dopamine agonists (which lower circulating levels of prolactin) reduced the interest of oncologists concerning a potential role of prolactin in the development of breast cancer. Within the last few years however, with the advent in molecular biology several studies have supported the role of prolactin in breast cancer.

Recent studies have reported that prolactin is also synthesized and secreted by mammary epithelial cells. There is expression and up regulation of prolactin receptors in tumor breast cell lines.[26] There is an autocrine / paracrine loop of action of prolactin to promote breast cancer. Prolactin also promotes

metastasis by inducing cell proliferation, altering Stat 5 levels of phosphorylation, changing the expression of human Prolactin receptor isoforms, tumour vasularizaton and cell motility.[27] Also studies show that prolactin receptor antagonists inhibit the growth of breast cancer cell lines.[28]

The other mechanisms such as increased expression of class II HLA antigen and up regulation of the breast cancer susceptible gene BRCA 1 by prolactin in breast cancer cell lines have been suggested for the tumorigenic effect of prolactin.[29,30]

Though in-vitro studies confirm the important role of prolactin in tumors of breast, results showing the same in-vivo are still awaited.

Conclusion

This study has shown a significant elevation of prolactin level in patients with carcinoma breast when compared with the control group. Also it has shown that there is a significant association between increased prolactin level and carcinoma breast. Though the prolactin level is increased in 70.4% of carcinoma breast patients, consideration of whether the prolactin level could be used as an indicator for carcinoma breast requires a larger scale study. Even when high level of prolactin is found in patients with distant metastases progression, further follow up of the cases is required.

This study supports the previous investigations which showed that increased prolactin level is associated with breast cancer and warrants a thorough investigation in future to know whether the hormone prolactin is important in etiology of breast cancer, so that early diagnosis using prolactin level and treatment strategies against prolactin would prove useful in preventing and curing cancer breast.

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