INTERLEUKIN-IL-6 AS A PROGNOSTIC MARKER IN PREDICTING METASTATIC BREAST CANCER IN SOUTH INDIAN WOMEN.

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Abstract
Background: Cancer occupies second place in one among the leading causes of death, of which breast cancer stands most common in Indian women. Inflammation causes progression of tumour which is primarily induced by excess production of pro-inflammatory cytokines like interleukin-6 (IL-6). Therefore, measuring of these levels helps in prediction of cancer.
Aim: The aim of this study is to measure the levels of IL-6 in breast cancer patients and the control group in order to find its significance. **Materials and Methods:**6 ml of blood sample was collected from patient group (30) diagnosed with ductalcarcinoma of Breast and control group (30) of healthy women. Serum was separated from the whole blood by centrifugation at 3000 rpm for 15 min and collected into aliquots. IL-6 levels were analysed by Human IL-6-ELISA kit (Enzyme linked immunosorbent assay) ELABSCIENCE .The lipid profile is also determined by enzymatic kit method. **Results:**Serum levels of Il-6 was found to be significantly higher in breast cancer patients when compared with the normal subjects. P value less than 0.05 is considered as statistically significant. **Conclusion: B**y measuring IL-6 levels in breast cancer patients, it can be suggested that IL-6 can be considered as a prognostic marker in identifying breast cancer patientsof south Indian region.
**Keywords:**breast cancer; interleukin-6, biomarker

Introduction
Breast cancer is considered as the top ranked cancers in women according to World Health Organisation report 2018. [1] It is a typical heterogeneous disease with thousands of mutations. This diverse background in genetics is reflected by multitude of breast cancers, among othersoestrogen or progesterone status, expression of HER2 with varied treatment approach and prognosis. It is considered as a multifactorial disease attributed by internal and external factors like obesity, inflammation and lifestyle which are increasingly acknowledged[2] The existence of lifestyle network of diseases plays a key role in understanding the over presentation of diseases like postmenopausal breast cancer, cardiovascular diseases, depression and diabetes. [3] The underlying etiology behind these disorders include accumulation of fat, systemic/ chronic low grade inflammation and obesity that have a pivotal role in breast cancer pathogenesis.Among the variations of breast cancer types, ductal carcinoma accounts for most common type which is associated with inflammation. Chemokines and cytokines attract the cells involved in inflammation which may contribute to the progression of breast cancer. The major cytokines involved are interleukin- 6 (IL-6) and interleukin -8 (IL-8) which initiates the carcinoma progression. [4]Between them Interleukin-6 (IL-6) is identified as an important player in inflammation and obesity.

Interleukin -6 is a pleiotropic cytokine with a molecular weight of 21-28 kDa. IL-6,upon binding with membrane bound receptor IL-6 R activates classical signalling pathways. It is a mediator of inflammation and functionally promotes several pro activities that increase growth, survival and activation of stromal fibroblasts. It is also involved in regulation of haematopoiesis, immune response, lipid metabolism and mitochondrial activity. The association in between cancer and inflammation is strongly reflected by Il-6 levels which promote multiple signalling pathways that include apoptosis, proliferation, invasiveness and metastasis followed by metabolism. [5]

Along with the levels of IL-6, lipid profile is also investigated as several studies have found the changes in serum lipids in cancer patients. The literature proves that patients with different kinds of cancer have noted with changes in concentration of serum lipids especially in breast cancer. The changes in lipid profile leads to enhanced production of tumour necrosis factor (TNFa) by macrophage activation [6]. In recent times, the identification of serological prognostic and biomarkers detection have gained much importance in search for disease onset, progression and therapeutic response. Therefore in the present study, we hypothesized that levels

of IL-6 along with lipid profile in tumour microenvironment measured in serum might be considered as prognostic marker for cancer.
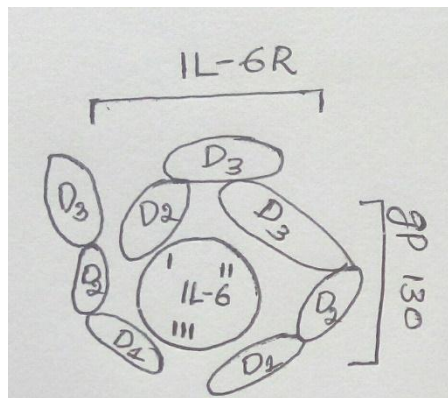
Fig.1 Top view of IL-6 complex

Need for the study: According to WHO report about 9.6 million people are estimated to die from cancer in 2018. Upon that Breast cancer has become more prevalent in women both in terms of incidence and mortality. Hence identification of it at early stages will be of great importance.

Materials and Methods

Sample Collection

Patients arriving to outpatient department of Saveetha Institute of Medical Sciences diagnosed with breast cancer were recruited for the present study. Patient fitting into inclusion criteria were categorised into case group and patients falling under exclusion criteria were categorised as control group. A total of 30 breast cancer cases and 30 healthy individuals were included in the present study. The age group of the samples ranged from 35 to 65 years who had no prior treatment. 6 ml of whole blood was collected from patient group and control group. Serum sample was separated from cases and controls prior to treatment and samples were stored at -80^0C until analysis. The study is conformed to the ethical standards of World Medical Association and was approved by Ethics committee of Saveetha University. Informed consent is obtained from all the patients recruited in the study.

Measurement of serum cytokine levels

Levels of IL-6 in the patients serum was determined by enzyme linked immunosorbent assays (ELISAs) according to the instructions given in the manufacturer's protocol of kit manufactured from Elabscience.Briefly, diluted 100µl serum samples were diluted and added onto 96 well plate and incubated for 90 min at 37^0C by sealing the plate with sealer. Then the liquid was removed from all the wells and 100µl of Biotinylated detection Ab working solution was added to each well, mixed and incubated for 1 hour at 37^0C. The complete content in the plate was discarded and washed with 350µl of wash buffer and the plate was pat dried and 100 µl of HRP conjugate working solution was added to all the wells, sealed and incubated for 30 min at 37^0C. The plates were then decanted and were washed again with wash buffer and 90µl of substrate reagent was added and incubated for 15 minutes at 37^0C. Then 50 µl of stop solution was added to each well and then the absorbance was read at 450 nm within 15 minutes. The mean absorbance was calculated for samples, controls and standards. The results obtained were tabulated and calculated according to the protocol.

Measurement of Lipid Profile

The lipid profile tests like Total Cholesterols (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) were analysed in both cases and control samples by using enzymatic kits.[7,8]

Statistical analyses

The values of cases and controls were used for analysing means and standard deviations. Theobtained data was evaluated by using Student's t-test for the patient group and the control group and p value < 0.05 is considered as statistically significant.

Results

Out of 30 cases and 30 controls, the estimated levels of IL-6 along with lipid profile parameters were significantly higher in cases than in controls. The mean and standard deviation values of cases and controls were calculated and results were depicted in the table 1. The P value was found significant for all the tests which shows that there is a strong co-relation between the breast cancer and the associated test parameters.

Table 1: Showing the estimated mean and standard deviation values of Cytokine and Lipid Profile values for Controls and Cases (Breast Cancer).

Test Parameter	Controls	Cases	P value
IL6	10.50±6.791	17.13±10.167	0.004
Cholesterol(mg/dl)	154.50±27.0	230.83±54.13	0.000
HDL(mg/dl)	72.93±15.51	31.80±5.690	0.000
LDL(mg/dl)	92.40±21.56	136.37±36.88	0.000
VLDL(mg/dl)	19.53±8.784	54.53±12.697	0.000
Triglycerides	91.43±23.88	190.83±37.49	0.000
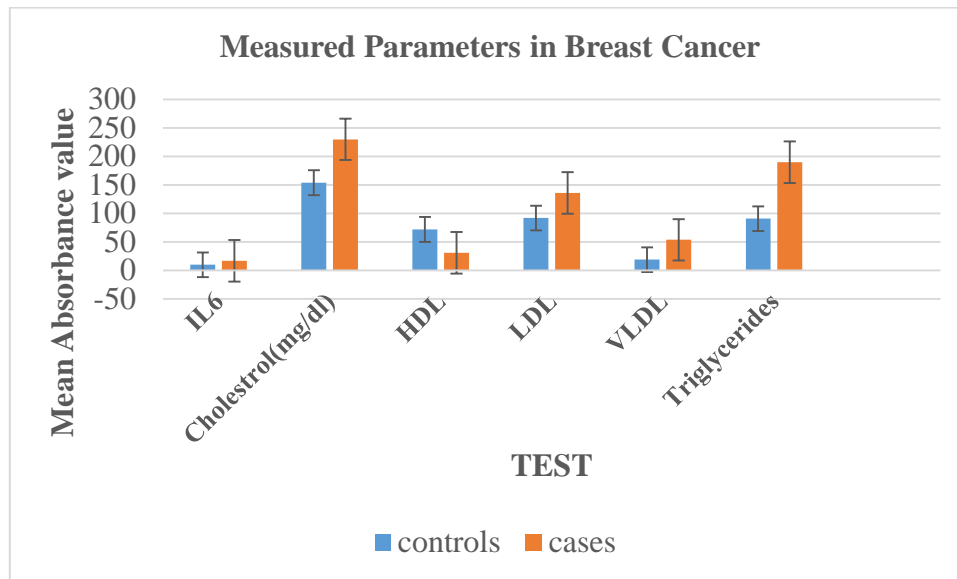
Figure 1: Histogram of tested parameters

Discussion

Breast cancer is currently a major diagnosed cancer in women worldwide. The treatment options include surgery, radiation therapy and chemotherapy which results in increased survival rate. The complete diagnosis can increase burden on both patient and health careprovider. On the other part, over treatment can incur drastic side effects on the patients. In recent times, prognosis and prognostic tumour burden relying on patients risk is focussed.Besides the parameters used for predicting therapeutic response and efficacy is in demand to stop over diagnosis and treatment.

As breast cancer is with cellular and molecular heterogeneity, patients with disease of same stage and grade might respond to varying therapy. Advances in gene expression provides a new way for predicting early stage cancer. [9] However, gene expression studies depend on tissue specimen gene expression based prediction develop limitations. One such limitation is that gene expression based approach which does not allow uninterrupted patient monitoring and assessment via treatment and surgery. This limitation might be overcome by inexpensive serum based tumour markers for disease progression and treatment. Hence we hypothesised whether serum could provide critical outcome and assist in breast cancer management. The study results revealed highest levels of IL-6 correlated with increased risk of early diagnosis compared with lower level. This results remained significant predictor of breast cancer and indicate measurement of serum IL-6 might be clinically useful marker to be added to the diagnostic marker lists of breast cancer.

Histological diagnosis of cancer has much difficulty because of cyto-morphological changes. Hence, determining serum markers for many cancers are under investigation. [10] In patients with breast cancer of South India , the measurement of IL-6 is not reported previously which is reported in the present study. Generally, tumour growth is associated with unbalanced cell proliferation and alterations in the growth regulatory cytokines. The study results presented that serum IL-6 is a prognostic marker in the untreated breast cancer studies. The levels of IL-6 in colorectal cancer correlates with the tumour proliferative status.[11] IL-6 is produced by variety of cell-lines of tumour associated with cell

proliferation, upregulation of anti-apoptotic proteins and induction of pro-angiogenic cytokines. [12, 13,&14]

Literature has shownimportance of Il-6 in processes associated with angiogenesis. [15, 16] In another in vitro model it was proved that cell proliferation is increased after adding IL-6. [17, 18] In vivo studies have proved that when IL-6 is transfected into human tumour cells it exhibited diminished tumour growth. These observations rely on the type of experimental model used. [19] These results present important observation of IL-6 in the progression of breast cancer similar to the data obtained in patients suffering from other kinds of cancer like mesothelioma, renal cell cancer and prostate cancer. [20,21,22, 23 &24]

Breast cancer is diagnosed for every one of three women.[25] The tumour grows slowly and becomes a lump and stands upto 10 years. Hence, early diagnosis is the best way to reduce the risk of breast cancer.[26] Therefore along with IL-6, the detection of lipid profile helps in adding support to IL-6 as a prognostic marker. The study findings proved that TC, HDL, VDL were found tobe increased in breast cancer patients which are in agreement with Florenza et al and Michael et al. The findings were also in disagreement with results of Patricia et al who stated that HDL levels were significanty reduced. [27,28,29] From the obtained results of the study, it was found that IL-6 and Lipid profile were found tobe significantly increased in breast cancer patients than in controls. The study findings were in agreement with the findings of Tornberg et al. [30]

Conclusion

The levels of IL-6 and Lipid profile were found tobe significantly high in breast cancer patients than in controls which proves that IL-6 can be used as a predictive marker in early diagnosis of breast cancer in South Indian population.

Conflicts of interest: Authors declares that they have no conflicts of interest.

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