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ROLE OF HEPATOCYTE GROWTH FACTOR IN INVASIVE BREAST CARCINOMA

Pathology

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ABSTRACT

Background: Breast cancer is the most frequently occurring cancer in women. It has been reported Hepatocyte Growth Factor(HGF) is the cause of many biological events of cancer like cell proliferation, movement, invasiveness, angiogenesis and morphogenesis. Estimation of serum HGF in many other solid carcinomas has indicated to be a good prognostic marker.

Objectives:

- To estimate serum HGF level in breast carcinoma.

Methods: Pre-operative estimation of serum HGF was carried out in patients with invasive breast carcinoma undergoing surgery. Serum samples from normal women and benign breast disease with age and sex-matched volunteers were used as control samples. **Results:** Serum HGF was significantly elevated preoperatively in invasive duct carcinoma cases as compared to benign breast disease and normal control samples(p-value<0.0001). **Conclusion** Thus the preoperative level of serum HGF has reflected the severity of invasive breast cancer in our study and is useful to pick up the high risk patients for more aggressive treatment.

KEYWORDS

Breast carcinoma, Serum HGF, ELISA, benign breast disease.

INTRODUCTION

Growth Factor

Growth factors are cytokines acting through specific cell-surface receptors, which results in normal homeostasis by bringing intricately balanced interactions between cells and the network of secreted proteins known as the extracellular matrix[1].

Growth factor activity is receptor mediated action which influence the expression of genes which can either promote entry of cells into the cell cycle, relieve blocks on cell cycle progression, prevent apoptosis or enhance biosynthesis of cellular components (nucleic acids, proteins, lipids, carbohydrates) required for a mother cell to give rise to two daughter cells.

Uncontrolled proliferation can result when the growth factor activity is dysregulated, or when its signaling pathways are altered to become constitutively active. Thus, many growth factor pathway genes are proto-oncogenes; gain-of-function mutations in these genes can convert them into oncogenes capable of driving unfettered cell proliferation and tumor formation.[2]

Hepatocyte Growth Factor

Hepatocyte growth factor is one of the growth factors which have many functions. Hepatocyte growth factor (HGF) was first identified in 1984 and 1985 and purified as a potent mitogen of primary cultured hepatocytes.

Structural characteristics of HGF

Molecular cloning revealed that it is a heterodimeric molecule composed of a 69-kDa alpha-chain and a 34-kDa beta-chain. The alpha-chain contains an N-terminal hairpin domain and subsequent four-kringle domains, and the beta-chain contains a serine protease-like domain with no enzymatic activity.

HGF was originally identified both as a mitogen for parenchymal liver cells and as a fibroblast-secreted protein responsible for the scattering of polarized epithelial cells (hence the alternative name, scatter factor) in 1985 and purified in 1989. Subsequent characterization revealed scatter factor to be identical to HGF.[3]

HGF is synthesized and secreted as a biologically inactive single-chain precursor form; further processing by serine proteases into the two-chain form is coupled to its activation. Serine proteases responsible for the activation of HGF include HGF activator or HGF-converting enzyme and urokinase-type plasminogen activator (uPA).

The receptor for HGF was identified as a c-Met proto-oncogene product. The c-Met receptor is composed of a 50-kDa α-chain and 145-kDa β-chain. The α-chain is exposed extracellularly, while the β-chain is a transmembrane subunit containing an intracellular tyrosine kinase domain.[4]

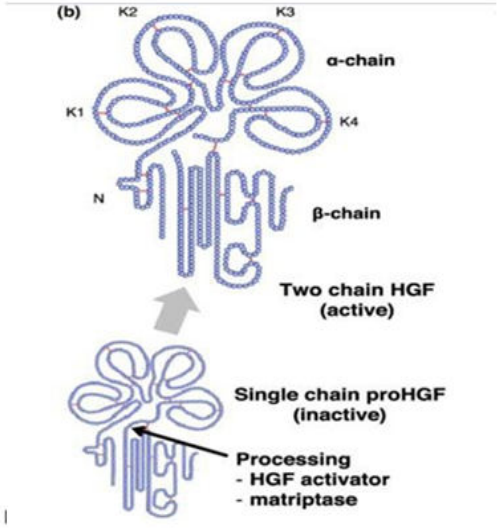


Figure No 1 Structure of HGF[4]Mechanism of Action of HGF

Binding of HGF to the c-Met receptor induces activation of tyrosine kinase, an event that results in subsequent phosphorylation of C-terminally clustered tyrosine residues. Now with considerable evidence it is known that intracellular signalling pathways driven by HGF-c-Met receptor coupling lead to multiple biological responses. These include motogenic (enhancement of cell motility), morphogenic, neurite extension and anti-apoptotic activities.[5]

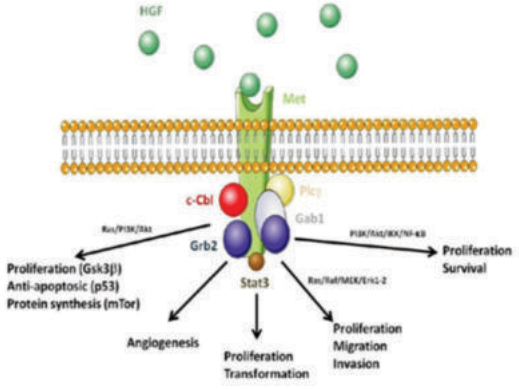


Figure No 2 : Schematic Representation And Mechanism Of Action Of HGF And Its Pathways[6].

In general, the c-Met oncogene is expressed in epithelial cells, whereas the ligand is expressed in the surrounding mesenchyme, providing a mechanism for the epithelial– mesenchymal inductive processes during development.[5] Many other well-established pathways include the Rho/ Rac pathway, the phosphoinositide 3 kinase pathway, Wnt signalling, the Grb2 pathway, β-catenin mediated pathways through which HGF action is mediated for which still more research evidence is needed. [7]

Role of HGF in Morphogenesis

HGF was identified as the fibroblast- derived growth factor that was both necessary and sufficient to stimulate epithelial cells derived from a variety of different organs to form tubule-like extensions when seeded in three-dimensional matrices (so-called 'branching morphogenesis')[8]. In one in-vitro assay, HGF stimulates some cells in the cell clusters to rearrange their cell polarity and extend processes. This is followed by the formation of cell chains that lengthen and thicken, re-establish cellular polarity and develop small lumens that coalesce to form a continuous lumen.[9] For example in the mammary gland, elevated expression of endogenous c-Met and HGF correlates with stages of active tubulogenesis, expression being high through early pregnancy but virtually absent during late pregnancy and lactation when alveologenesis and gland differentiation take place.[10]

Role of HGF in Angiogenesis

Growing cancers stimulate neoangiogenesis, during which vessels sprout from previously existing capillaries. HGF has angiogenic action. HGF acts directly on endothelial cells, inducing proliferation, migration and on tumor cells, in which it triggers angiogenic switching by up-regulating the expression of the proangiogenic factor VEGF and down-regulating the expression of TSP-1, an angiogenesis inhibitor. Inhibitors of the HGF–cMet pathway would therefore have the potential to both block VEGF expression and boost TSP-1 levels while simultaneously interfering with invasion and metastasis.[11]

Anti-apoptotic role of HGF.

Apoptotic events are one of the key pathogenic causes for the manifestation of organ injury. HGF prohibits apoptotic signals via inhibition of caspase-3 activity or induction of anti-apoptotic molecules, such as Bcl-xL.[12] HGF also prohibits Fas-mediated apoptosis signals via sequestration of Fas and c-Met on cell surfaces. [13]

HGF and cMet regulate both morphogenic and tumorigenic phenotypes:

HGF is a potent inducer of EMT in many epithelial systems, like other growth factors eg., FGF and EGF . HGF is a multi-functional cytokine that stimulates morphogenesis, cell survival , motility, invasion, and metastasis. In normal mammary development, HGF is produced by mesenchymal cells and along with other growth factors and the stromal protein epimorphin stimulates tubulogenesis in a tightly controlled paracrine manner.

Over-expression of HGF and Met occurs in many types of invasive cancers, including breast carcinomas . A shift from transient activation of Met to sustained high levels of c-Met activation and co-operativity with other RTKs can cause a switch in the HGF response from morphogenesis to tumorigenesis.[14]

Role of HGF in Breast Carcinoma:

HGF is synthesized in the mammary stroma, probably by fibroblasts, and acts on receptor-expressing ductal epithelial cells. This concept strongly suggests that HGF/SF c-Met signalling is a classical epithelial–mesenchymal inductive pathway that is important for ductal morphogenesis in the mammary gland.HGF may co- operate in the regulation of the migration of epithelial cells across the fatty stroma by altering integrin–matrix signaling locally. High levels of HGF and Met expression in breast carcinoma cells is a possible independent predictor of recurrence and shortened survival in breast cancer patients.[15]

In normal breast epithelium and ductal carcinoma in situ (DCIS), strong expression of E-cadherin with accentuation at cell–cell contacts is evident . In contrast, decreased expression of E-cadherin frequently occurs in invasive ductal carcinoma (IDC), while c-Met expression is relatively consistent throughout, and most intense in IDC. Sustained activation of HGF-cMet signalling is associated with dissociation of cadherin-based adherens junctions, followed by loss of cadherin expression. The modulation of adherens junctions by HGF–cMet involves phosphorylation of β- catenin, leading to its reduced affinity for the E-cadherin complex and subsequent degradation . HGF disrupts adherens junctions and promotes cell dispersal, so stimulating invasive capacity.[16,17]

Hgf And Its Signalling Complex As Therapeutic Targets

Targeting HGF, HGF receptor, and signalling events has been an attractive option for cancer therapy. Therapeutic approaches have been attempted by developing tools against: HGF (neutralizing antibodies, antisense oligonucleotides, ribozyme, short interfering RNA (siRNA), and HGF regulators including HAIs), cMet (HGF antagonists, antibodies, small molecules, antisense, ribozymes, siRNA, and non-specific inhibitors), cMet signalling events (coincident with anti-cMet methods), and HGF activation inhibitors. These therapeutic approaches are largely in the development phase, with a small number—mostly non-specific inhibitors to cMet— now in early clinical study.[18]

MATERIALS AND METHODS

Methodology Source Of Data

The present prospective study was undertaken in the Department of Pathology, JSS Medical College and Hospital, Mysuru.

Type Of Study Descriptive study

Inclusion criteria

Histopathologically confirmed invasive duct carcinoma of breast were included in the study.

Exclusion criteria:

Breast carcinoma other than invasive duct carcinoma, diagnosed on core biopsy and patients having other known malignancy were excluded from the study.

Method Of Collection Of Data

- Clinical details were collected from the patients through structured questionnaire.
- Venous blood samples were collected preoperatively; serum was separated and stored at temperature -80°c from patients with breast carcinoma undergoing surgery.
- Serum samples from normal women and benign breast tumor patients with age matched volunteers were used as control samples.
- Serum HGF was estimated using HGF ELISA kit (Thermofischer, Sandwich enzyme method)
- Gross and microscopic features of the mastectomy specimen were studied.

PROCEDURE:

Serum HGF assay by ELISA

The estimation of serum HGF was done by using HGF ELISA kit (Thermofischer, Co, Ltd.) using sandwich ELISA method with assay sensitivity of <10pg/ml. To Anti- HGF antibody precoated microplates wells, serum samples of the cases, controls and standards were pipetted. During the first incubation, the protein antigen bounds to the capture antibody. After washing, a detection antibody was added to the wells, and this antibody bounds to the immobilized protein captured during the first incubation. After removal of excess detection antibody, streptavidin peroxidase (HRP) conjugate was added which bounds to the detection antibody. After a third incubation and washing, to remove the excess HRP conjugate, a substrate solution was added and was converted by the enzyme to a detectable color signal. The intensity of this colored product was directly proportional to the concentration of antigen present in the original specimen. Values were read at 450nm OD. Standard curve is obtained by using a software from which the concentrations of the samples and standards are obtained.

Histopathological Examination:

Mastectomy specimens were fixed in 10% neutral buffered formalin within one hour of resection. They were examined grossly according to standard guidelines and noting all the important parameters, specimens were fixed for 24-48 hours. Sections were taken from representative sites, processed in automated tissue processor followed by paraffin embedding and staining with Haematoxylin & Eosin.

Sections were studied to establish final histopathological diagnosis.

Statistical analysis

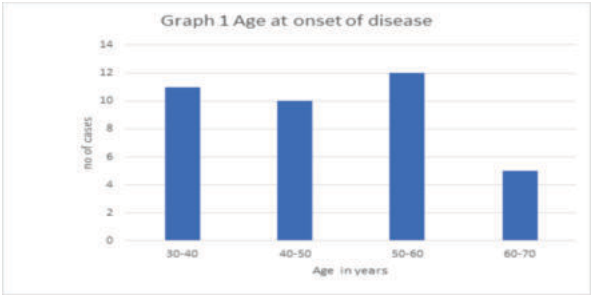
The results were statistically analysed using SPSS version 22.

Statistical Methods
1 Descriptive statistics
2. Chi-square tests
3. Contingency table analysis using SPSS for Windows
4. P values <0.05 were considered statistically significant

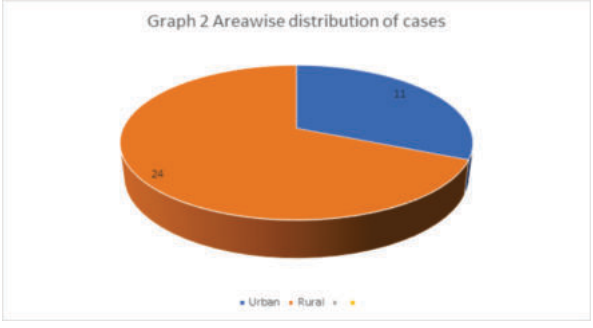
RESULTS AND OBSERVATION

The present study was undertaken from September 2016 to July 2018 in the Department of Pathology, J.S.S. Medical College and Hospital, Mysuru. A total number of 35 invasive ductal breast carcinoma as cases; 9 benign breast disease and 8 normal samples as controls were evaluated.

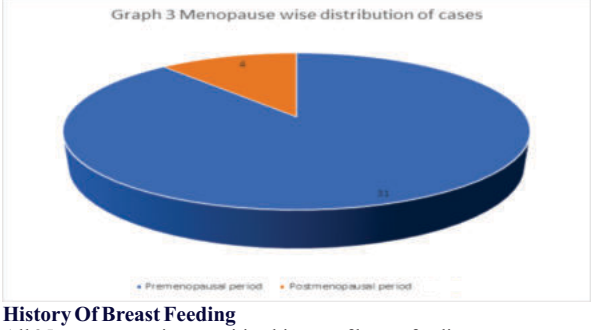
Age At The Onset Of Disease:
Total number of cases studied were 35, age range varied from 33-65years with a mean age of 45±15years and maximum number of cases were in the age group of 50-60 years accounting for 34.2% . (Graph 1)



Area Wise Distribution Of Cases:
Out of 35 cases, majority of cases were from rural area (68.57%), rest being distributed in urban area(31.43%) (Graph 2)



Relation With Menopause:
Out of 35 patients, 31 (88.57%) were in premenopausal period and the remaining 04(11.43%) in postmenopausal period. (Graph 3)

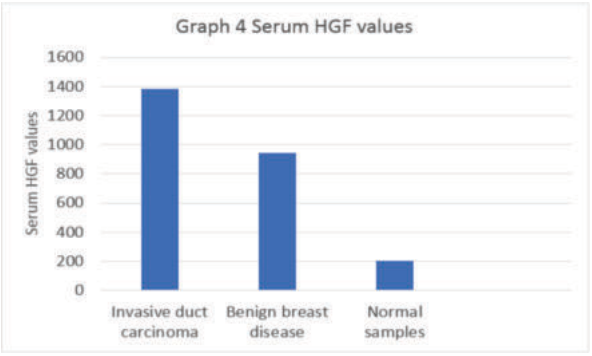


History Of Breast Feeding
All 35 cases were given positive history of breast feeding

Serum HGF Values:
Serum HGF values in Invasive duct carcinoma was in the range of 936-2731pg/ml with a mean of 1386pg/ml. and other values as depicted in the Table No 01. The p value is <0.0001 which shows statistical significance in the present study. (Table No 01)

Table No 01. Serum HGF Values

Category	No of cases	Serum HGF pg/ml(range)	Mean value (pg/ml)	p-value
Invasive duct carcinoma	35	936-2731	1386	<0.0001
Benign breast Disease	9	726-1556	944	
Normal serum samples	8	160-481	337	



DISCUSSION

Introduction
Breast cancer is the second most common cancer among women in the world. In order to reduce the morbidity and mortality associated with breast cancer, there is a need to search for a biomarker to diagnose at early stage and to predict its prognosis.[19] HGF known to be identical to scatter factor plays an important role in various stages of cancer progression including cell proliferation, movement, invasiveness, morphogenesis, and angiogenesis.[20] Estimation of serum HGF in many other solid carcinomas has indicated to be a good prognostic marker. This study was conducted to estimate the serum levels of HGF in invasive ductal carcinoma of breast to know its significance.

Age Distribution:
In the present study, the age range of the patients with invasive breast carcinoma varied from 33 years to 65 years with a mean age of 45.63±15 years. Mean age of other studies were variable and the results of present study was comparable with H A Attar et al[21] as shown in the Table No 02.

Table No 02 Comparison of Age Distribution with Other Studies

Study	Age range (Years)	Mean (Years)
H.H. Ahmed et al [24]	23-56	36±10
H A Attar et al [21]	30-56	47.5±10
SM S Chen et al [20]	31-84	50.5±20
T Tanaguchi et al [22]	21-88	50.9±30
R Kucera et al [23]	28-84	61.8±20
Present study	33-65	45±15

Relation With Menopause:
Out of 35 patients, 31 (88.57%) were in premenopausal period and the remaining 04(11.43%) in postmenopausal period. This was comparable with the study done by H H Ahmed et al 24, where maximum cases (72.73%) were in premenopausal period.

Association Of Age With Serum Hgf And Comparison With Other Studies:
In the present study the serum HGF values when compared among different age groups show no statistical significance (p-value-0.265) which was similar to H A Attar et al21(p-value-0.186) and SMS chen et al20 (p-value-0.545) findings. The mechanisms by which serum HGF is released in different age groups are unclear but found to be regulated by many other factors like IL-1β, prostaglandin E2, heparin, bFGF, EGF and PDGF 4

Serum HGF levels:
There was a significant increase in preoperative serum HGF levels of invasive ductal carcinoma patients as compared to benign breast disease and normal women. Serum HGF levels obtained among carcinoma patients was in the range of 936-2731pg/ml, mean value being 1386pg/ml as compared to benign breast diseases with a range of 726-1556pg/ml, mean value =944pg/ml and normal samples ranged 160-481pg/ml, mean value=337g/ml. The p value is <0.001 which shows statistical significance in the present study. These values were comparable with the following studies.

Table No 03 Comparison of serum HGF values with other studies

Study group	Range of Serum HGF (pg/ ml) in carcinoma	Mean (pg/ml)	p-value	No of cases
H A Attar et al [21]	356-2352	1073	0.000	38
H H Ahmed et al [24]	-	1198	0.026	44
T Tanaguchi et al [22]	-	410	-	134

S M S Chen et al [20]		529	<0.001	124
R Kucera et al [23]	262- 21838	3370	0.0016	89
Present study	936-2731	1386	<0.05	35

HGF is thought to be a stromally derived paracrine growth factor in breast cancer, because human cultured breast cancer cells express the HGF receptor, c-Met, but not to produce HGF by themselves 25. The increase in HGF could be explained by overstimulation of the cells that secrete HGF through auto secretion or mutation and aberrant regulation of the HGF-cMet signalling pathway or an imbalance between HGF activators and inhibitors.26 In Study done by Tanaguchi et al, it was found that serum HGF levels was due to the presence of tumor and removal of the primary tumor clearly decreased the serum HGF level in primary breast cancer patients.22 Several growth factors or cytokines including tumor necrosis factor alpha, interleukin 1 , and transforming growth factor 3 are known to be responsible for the production of HGF in stromal cells.27 This result was consistent with SMS Chen et al20 and H H Ahmed et al24 who detected that preoperative serum HGF levels were significantly elevated in the patients compared to those in control (p-value-0.026). Thus, the preoperative level of serum HGF may reflect the severity of invasive breast cancer and may be useful to pick up the higher risk patients for more aggressive treatment.

CONCLUSION

The present study was a descriptive type of study conducted at JSS hospital, Mysuru. Preoperative serum HGF estimation was done in 35 cases of invasive ductal carcinoma using sandwich ELISA assay which were compared with benign breast disease and normal samples as controls. The study showed a significant increase in serum HGF levels in invasive ductal carcinoma as compared to benign breast disease and normal samples and there was statistical significance. This was comparable with various previous studies. The increase in HGF could be explained by overstimulation of the cells that secrete HGF through autosecretion or mutation and aberrant regulation of the HGF/cMet signalling pathway or an imbalance between HGF activators and inhibitors. In tumors, HGF disrupts adherens junctions and promotes cell scattering, dissociation and disruption of E-cadherin mediated breast cancer cell adhesion. Following HGF stimulation, the CrkII and CrkL adapter proteins are recruited to cMet-dependent signalling complexes. This appears to be the key to the breakdown of adherens junctions, the spreading of epithelial colonies and the formation of lamellipodia in response to HGF.

Some of the previous studies have shown association with the above parameters which may be due to that the tumor microenvironment promotes the production of HGF variants and could disrupt or modify HGF/cMet function during tumor progression. As various fragmented forms of HGF and soluble cMet exists in the sera of cancer patients, the identity of the HGF measured is probably different among ELISA and the correlation between serum HGF level and clinical features could be variable.

Thus the preoperative level of serum HGF has reflected the severity of invasive breast cancer in our study and is useful to pick up the higher risk patients for more aggressive treatment.

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