RESEARCH COMMUNICATION

Hepatocyte Growth Factor in Egyptian Females with Breast Benign Lumps and Cancers

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Abstract

Background: Hepatocyte growth factor(HGF) also known as scatter factor (SF) and its receptor c-met play important roles in mammary differentiation and have been implicated in mammary carcinogenesis. Objective: Estimation of the plasma level of HGF in females with benign breast lumps or breast carcinomas and correlating levels with important prognostic parameters. Subjects: Sixty eight adult premenopausal females were divided into control group of fifteen healthy volunteers and fifty-three patients subdivided into fifteen with benign breast lumps and thirty-eight with breast carcinomas. Methods: A thorough clinical examination, plain chest x-rays, ultrasonography of the abdomen and pelvis, pre- operative fine needle aspiration cytology, estimation of fasting serum glucose, urea, creatinine and uric acid levels, alanine aminotransferase activities, C-reactive protein, HGF level and histopathological examination of the breast masses were performed. Results: Significant increase in HGF levels was found in patients with benign breast lumps and in breast cancer patients when each was compared to controls and when cancer patients were compared to the benign breast lumps group. Conclusion: The serum level of HGF is an independent prognostic indicator for breast cancer.

Keywords: Breast lumps - hepatocyte growth factor - metastasis

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Introduction

Most breast cancers evolve over a long latent period by several processes which require cell growth (mitogenicity), migration (motogenicity) and differentiation which remain mostly undetermined (Cao et al., 2006). Growth factors (GFs) are polypeptide molecules that bind to receptors on cell surface (Favoni et al., 2000). Hepatocyte GF, also known as scatter factor, Hepatopoietin A, lung fibroblast derived mitogen and Met -ligand , was originally discovered with liver regeneration (Liu et al., 2006).

Hepatocyte growth factor is synthesized as biologically inactive chain precursor (728 amino acids called pro -HGF) (Parr et al., 2004) which requires proteolytic processing, by various serine proteases to generate active two - chain form (HGF) in vitro (Ohnishi et al., 2006). It is primarily expressed in stromal cells and weakly in epithelial and endothelial cells (Peruzzi et al., 2006) .Activator inhibitor type -1 and type - 2 are two serine protease inhibitors with the ability to bind HGF activator and thus block its HGF - activating properties (Cao et al., 2006).

Hepatocyte GF promotes mammary ductal morphogenesis and in maintaining ductal spacing (Pollard et al., 2001) and is also expressed in " benign breast disease" detected as incidental microscopic findings (Guray et al., 2006).

Fibrocystic changes are the most frequent benign disorder of the breast (Guray et al., 2006). Cysts contains a variety of biologically active substances including steroids, cytokines, growth factors as well as proteins (Celis et al., 2006).

Previous workers found that expression of HGF and its receptor c - Met is correlated with breast carcinoma progression (Beviglia et al., 1999).

The study was done to estimate the serum level of HGF in females with benign breast lumps and females with breast carcinoma and correlating its level with the prognostic parameters .

Materials and Methods

The study involved sixty eight adult premenopausal females divided into three groups : Group I ; fifteen normal healthy volunteer females, Group 2; included fifteen females with benign breast lumps and Group 3 included thirty eight females with breast carcinoma . Pregnancy or intake of contraceptive hormonal therapy at the time of the study were excluded.

To all studied subjects, thorough clinical examination was done, plain x-ray chest, ultrasonography of the abdomen and pelvis and laboratory investigations included fasting serum (Burtis et al., 2006) glucose, urea, creatinine, uric acid, alanine aminotransferase and alkaline

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phosphatase activities. Serum HGF was quantitatively determined using a solid phase sandwich ELISA from Biosource human (HU) Kit (Cat number KAC 2212/ KAC 2211) USA (Sheen et al., 2005). Histopathological examination: Preoperative as fine needle aspiration cytology and post operative for breast mass after surgery were performed.

Data were analysed using SPSS soft ware package, version 11.5 (SPSS, Chicago, IL, USA) for statistical analysis. Presentation was in form of median and range for such non parametric data .

Results

A significant increase in age was found in breast cancer patients group when it was compared with its value in patients with benign breast lumps group .

As regard subjects with benign breast lumps (n=15), nine patients (60%) had fibroadenoma , four patients (26.6%) had fibrocystic disease of the breast and two (13.4%) patients had chronic non specific inflammation.

All breast cancer patients (n=38); (100%) had infiltrating ductal carcinoma .

Thirty six patients (94.73%) had IDC grade II, two (5.26%) grade III.

Nine patients (23.7%) had tumor size ≤ 2cm in its biggest diameter (T1), twenty two patients (57.9%) had tumor size >2cm and ≤ 5cm (T2) and seven patients (18.4%) had tumor size > 5cm in the biggest diameter (T3) . According to tumor size and lymph node status , the patients were divided into three stages, five patients (13.2%) were stage I , eleven patients (28.9%) were stage II and twenty two patients (57.9%) were stage III.

Thirty two patients (84.2%) were (ER) positive and only six patients (15.8%) were (ER) negative . Twenty five patients (65.8%) were (PR) positive and thirteen patients (34.2%) were (PR) negative. Twenty patients (52.61%) were having prominent angiogenesis (>30/10 HPFS) and eighteen patients(47.4%) were having angiogenesis less than thirty (<30/10HPFS).

Table 1. Serum Levels of Fasting Glucose (FSG) , Urea (Ur) , Creatinine (Cr) , Uric acid (UA), Alanine aminotransferase (ALT) and Alkaline Phosphatase (AP) Activities and Hepatocyte Growth Factor (HGF) (pg/ml) in the Studied Groups

Group / Item		FSG (mg/dl)	Ur (mg/dl)	Cr (mg/dl)	UA (mg/dl)	ALT (U/L)	AP (U/L)	HGF (pg/ml)
Control group	Median	90	25	0.9	4.4	12	82	311
(group1)	Min-max	82-96	18-30	0.6-1.3	4-5.7	8-17	47-92	177-814
(n=15)								
Benign breast	Median	88	18	0.8	4.6	16	74	554*
lumps group	Min-max	80-100	11-32	0.7-1.0	4.1-5.7	5-30	49-95	279-1220
(group2)	p							0.002
(n=15)								
Breast cancer	Median	95*•	26•	0.9	4.45	13.5	81	1073.5*•3
patients group	Min-max	82-100	16-40	0.6-1.3	4-6	5-40	39-260	56-2352
(group3)	P	0.017	NS					0.000
(n=38)	P_1	0.009	0.005					0.000

*P=Statistical difference from group 1 (control subjects).; •P_1=Statistical difference from group 2 (subjects with benign breast lumps).; NS=No significant difference.

Table 2. Median (and Range) of HGF for 38 Cases of Cancer Breast Presented According to Age, Tumor size, Grade, Lymph Nodes Involvement, Stage, ER, PR and Angiogenesis

	Classification	No	Median	Range	P	P1	P2
Age	≤50	32	1121.5	356-2352			
	>50	6	948.5	638-1244	0.186		
Tumor size	T1	9	1006	706-1570			
	T2	22	1141.5	356-2244	0.728		
	T3	7	1222	744-2352		0.223	0.359
Grade	Grade Π	36	1073.5	356-2352			
	Grade Ш	2	872.5	491-1254	0.472		
Lymph node	LN=N0	11	1078	491-2532			
	LN ≤3	6	841	732-1286	0.159		
	LN>3	21	1118	356-2244		0.89	0.321
Stage	Stage I	5	1021	891-1570			
	StageΠ	11	1165	491-2352	0.955		
	StageШ	22	1093.5	356-2244		1.00	0.97
ER	positive	32	1045	356-2352			
	negative	6	1249	908-2244	0.262		
PR	positive	25	1078	356-2352			
	negative	13	1069	638-2244	0.890		
Angiogenesis	>30/10HPFs	20	966	356-2244			
	<30/10HPFs	18	1141.5	491-2352	0.483		

P= Statistical difference between first and second groups.; P_1= Statistical difference between first and third groups.; P_2= Statistical difference between second and third groups.

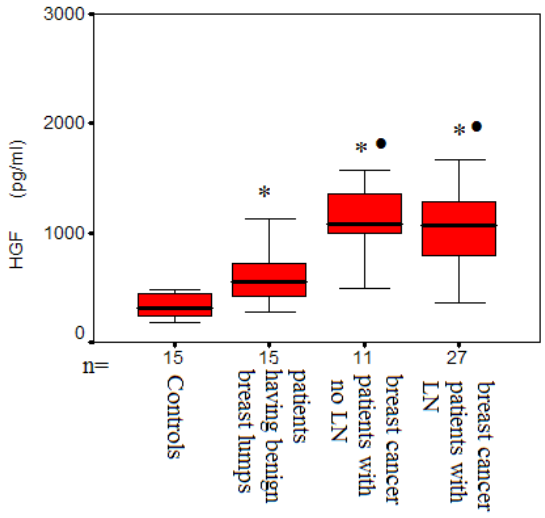

Figure 1. Box Plot Presentation of Serum Level of Hepatocyte Growth Factor (HGF) (pg/ml)

Table 1 shows serum level of fasting glucose (FSG), urea (Ur), creatinine (Cr), uric acid (UA), and serum alanine aminotransferase (ALT) and serum alkaline phosphatase (AP) activities and HGF (pg/ml) in the studied groups.

Significant increase in HGF level was found in patients with benign breast lumps group and in breast cancer patients group when each was compared with the control group.

A significant increase was also found in breast cancer patients group when compared to patients with benign breast lumps groups.

No difference was found between patients having breast cancer without lymph node metastasis and those with lymph node metastasis groups (Figure 1).

In breast cancer patients, no significant differences in serum level of HGF with any of the prognostic parameters (Table 2).

Figure 1shows box plot presentation of serum level of HGF (pg/ml) in control group, patients with benign breast lumps and in patients having breast cancer without and with lymph node metastasis.

Discussion

Benign breast lumps and carcinoma of the breast are biologically different diseases . Several polypeptide hormones and growth factors have been shown to play an active role in breast epithelial cell growth and development (Hondermarck et al., 2003). Genetic aberration in growth factor signaling pathways are strongly connected with lumps (Cao et al., 2006).

Hepatocyte growth factor which belongs to the plasminogen prothrombin gene superfamily (Cao et al., 2006), plays a role in mammary gland development (Pollard et al., 2001).

Previous workers found that in breast cancer deregulated HGF and c-Met signaling may be found (Bonine-Summers et al., 2007) .

In benign breast lumps group , the median level of HGF was 554pg/ml (279-1220pg/ml) vs 311pg/ml (177-814pg/ml) wich was significantly higher than its level in control group (p=0.002) (Table 1). This was not comparable with previous work (Sheen-Chen et al., 2005) that showed lower values.

In the present study ,HGF level in breast cancer patients group was 1073.5pg/ml (356 -2352pg/ml) (Table 1) which was comparable with results of previous workers (Taniguchi et al., 1995). However, Sheen-Chen SM et al., (2005) reported lower values. A significant increase in HGFlevel in whole breast cancer patients group when compared with control group and patients with benign breast lumps group (p=0.000 for both) (Table 1). This finding is in accordance with the results of other workers (Taniguchi et al., 1995; Tuck et al., 1996; Jin et al., 1997; ; Cianfrocca etal., 2004 , Parr et al., 2004; Sheen-Chen et al., 2005).

By immunohistochemistry, HGF concentration was significantly higher in breast tumor tissuesthan benign lumps and normal tissues . Previous workers found that HGFis higher in tumor tissues than in adjacent normal tissues (Yamashita et al., 1994; Nagy et al., 1996; Rahimi et al., 1998; Favoni et al., 2000; Parr et al., 2004) .

The increase in HGF, could be explained by: over stimulation of the cells that secrete HGF, or mutation and aberrant regulation of the HGF/Met signaling pathway or an imbalance between HGF activators and inhibitors.

In the present work, no significant difference was found in serum HGFlevel between patients having breast cancer with and without LNmetastasis (Figure 1). As HGF median value was 1069 pg/ml (356-2244pg/ml) in patients having breast cancer with LN metastasis group and was 1078pg/ml (491-2352pg/ml) in patients without LN metastasis group.

In breast cancer patients, no significant difference was found in HGFand age. Similar findings are found by Sheen-Chen et al., (2005) and others (Yamashita et al., 1994; Wei-Kai et al., 2007). Also, no significant difference was found between HGF level and tumor size like others (Taniguchi et al., 1995; Sung et al., 2003) and tumor grade as only two patients were grade III and the rest were grade II.

Higher serum levels of HGF in patients with high grade tumor which does not agree with our study were found (Taniguchi et al., 1995; Sheen-Chen et al., 2005).

No significant difference in HGF level and LN metastasis which was found by Sung et al., (2003) (Sung et al., 2003).

Serum level of HGF in the current work, showed no significant difference and the stage of cancer . However, previous workers found that HGF levels were higher with high- stage disease (Sheen-Chen et al., 2005) and in patients with liver metastasis and in patients with multiple metastasis sites (Taniguchi et al., 1995; Eichbaum et al., 2007) compared with benign breast disease .This was not found as our patients were seeking medical advice before this very late stage.

In the present study , no significant associations in HGF level and hormone receptors (ER and PR) were found which goes in agreement with other work (Sung et al., 2003) also between tissue level of HGF in breast

cancer tissue and hormone receptors (Yamashita et al., 1944; Wei-Kai et al., 2003).

In the present study, serum level of HGF did not show significant difference with angiogenesis. Previous study (Yamashita et al., 1944) found no significant associations. However, other workers (Taniguchi et al., 1995; Sheen-Chen et al., 2005) found that HGF stimulates tumor invasion and neovascularization. So it may play important roles in tumor growth and metastasis.

In conclusion, serum level of HGF in benign breast lumps and breast cancer patients showed significant increase when compared withcontrols. A significant increase in its level was found in breast cancer patients as compared to benign breast lumps groups. HGF is an independent prognostic marker in breast cancer. More studies are needed on HGF receptors and of HGF activators and inhibitors.

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