Possible effects of insulin-like growth factor-I, IGF-binding protein-3 and IGF-1/IGFBP-3 molar ratio on mammographic density: a cross-sectional study

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Summary

The purpose of this study was to examine the possible effects of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio on mammographic density and assess whether this relationship was similar in subgroups of pre- and postmenopausal women. A group of 341 Italian women of childbearing age or naturally postmenopausal who had performed mammographic examination at the section of radiology of our department a maximum three months prior to recruitment were enrolled. A blood sample was drawn for determination of IGF-1, IGFBP-3 levels and IGF-1/IGFBP-3 molar ratio was calculated. On the basis of recent mammograms the women were divided into two groups: dense breast (DB) and non-dense breast (NDB). To assess the association between mammographic density and IGF-1, IGFBP-3 and Molar ratio Student's t-test was employed before and after stratified by menopausal status. The analysis of the relationship between mammographic density and plasma levels of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio showed that IGF-1 levels and molar ratio varied in the two groups resulting in higher mean values in the DB group whereas IGFBP-3 showed similar values in both groups (DB and NDB). After stratification of the study population by menopausal status, no association was found. Our study provides strong evidence of a crude association between breast density, and plasma levels of IGF-1 and molar ratio. IGF-1 and molar ratio might increase mammographic density and thus the risk of developing breast cancer.

Key words: IGF-1; IGFBP-3; IGF-1/IGFBP-3; Molar ratio; Mammographic density; Breast cancer risk.

Introduction

Insulin-like growth factor-1 (IGF-1) is an essential growth factor for the regulation of proliferation and apoptosis in normal mammary cells [1, 2]. There is increasing evidence of the role of IGF-1 in the growth of tumors in a number of different cancers such as prostate, colon and lung cancer [3, 4] including breast cancer [2, 5, 6]. The IGF-1 mechanism of action in carcinogenesis and development of breast cancer is complex and multifactorial [1, 2, 5-11]. IGF-1 circulates in the blood and it has a very short lifetime in free form, approximately 12 minutes; its action is strongly influenced by the association with one of six existing different insulin-like growth factor binding proteins (IGFBPs) which increases its average lifetime to about 12 hours [1, 2]. Over 90% of IGF-1 in circulation is bound to form-3 of IGFBP (IGFBP-3). This complex remains stable in the blood due to the presence of a binding protein, specific protease inhibitor [1, 7].

In the extravascular space, the lack of this inhibitor allows specific metalloproteases to break the link between IGF and IGFBP-3 thus favoring the association between IGF and its specific cellular receptor (IGFR) expression in the breast tissue where IGF-1 fulfills its regulatory role [8].

In addition to the regulatory effect on the action of IGF-1 the IGFBP-3 present in the tissue also seems to directly promote cell apoptosis independently of IGF-1 [7-10]. Several studies have demonstrated an association between the IGF system and breast cancer risk in premenopausal women [6, 12-14], suggesting that IGF-1 might interact with the estrogen signal to increase cell proliferation [15, 16]. Other more recent studies have reported a possible association between the IGF system and carcinogenesis also in postmenopausal women [17].

The debate concerning the association between menopausal status, IGFBP, IGF-1 and breast cancer risk is therefore still open [18, 19]. On the other hand, breast density is currently considered as the strongest breast cancer risk factor [20]. Women with mammographic density ≥ 75% have a five-fold increased risk of developing breast cancer compared to women with fatty breast tissue and density < 5% [21-24].

Given the regulatory function of IGF-1on the proliferation of normal breast tissue, the question has been raised whether there is a possible association between IGF and breast density. Some authors have shown a significant association between IGF and breast density in premenopausal women [13, 15, 25-28]. The results reported in the literature related to postmenopausal women are still discordant, as most authors found no correlation between IGF and breast density [25-29], whereas some authors reported a weak relationship also in postmenopausal women [30] whether they were receiving hormonal therapy [31] or not [30].

The main objective of the present study was to analyze whether there is a relationship between plasma levels of IGF-1, IGFBP-3, IGF-1/IGFBP-3 molar ratio and mam-

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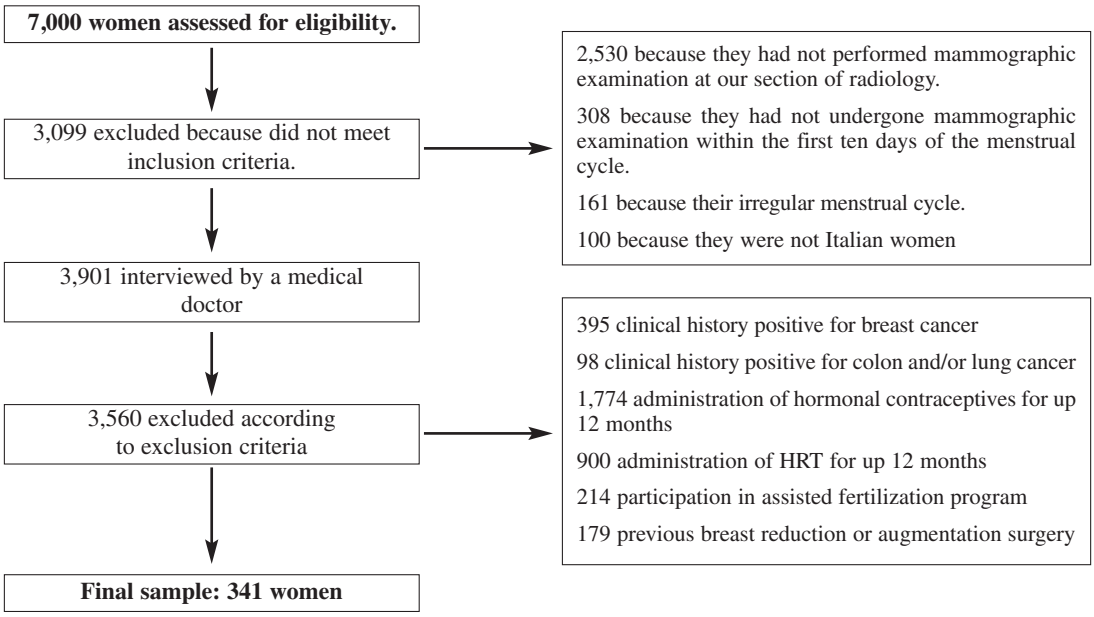

Figure 1. — Flow chart of the sampling strategy.

mographic density in a study population of Italian Caucasian women. The secondary objective was to assess whether this relationship was similar in subgroups of pre- and postmenopausal women.

Materials and Methods

Ethical approval for this single-center, prospective study was granted by the Medical Research Ethics Committee of our institution and written informed consent was obtained from all patients.

The sample was built up in the order of presentation and 7,000 women were selected among those who spontaneously turned to the Breast Care section of the Department of Obstetric and Gynaecological Sciences and Urological Sciences of the University of Rome "Sapienza" for a breast examination between March 2005 and March 2007.

According to the protocol we selected only Italian women of childbearing age (regular menstrual cycles during the past year) or naturally postmenopausal women (absence of menstrual cycles for at least 12 months) who had performed mammographic examination, negative for breast cancer pathology, at the section of radiology of our department maximum three months prior to recruitment. Premenopausal women were enrolled in the study only if they had undergone mammographic examination within the first ten days of the menstrual cycle.

After recruitment the women were interviewed by a medical doctor.

Women with a clinical history positive for breast cancer and/or for colon and lung cancer, administration of hormone therapy for up to 12 months before recruitment such as menopause hormone replacement therapy (HRT) and hormonal contraceptives, participation in assisted fertilization programs and previous breast reduction or augmentation surgery were excluded from the study.

The objective of the study was explained to all the selected subjects. The first phase of the study included signing of an informed consent form, collection of recent mammograms as well as drawing of blood samples for the evaluation of serum IGF-1 and IGFBP-3 and calculation of IGF-1/IGFBP-3 molar ratio.

Patients were divided into two groups: dense breast (DB) or non-dense breast (NDB) according to the mammographic parenchymal category assigned at the evaluation of the presented mammograms. Subsequently patients were stratified by menopausal status.

Mammographic classification

To determine the mammographic parenchymal category all mammograms were examined by three physicians (two radiologists and a gynaecologist and breast specialist) all blinded to the clinical data and to the classification already assigned. Particular attention was paid to the craniocaudal projections of both breasts and the distribution of glandular parenchyma was qualitatively evaluated in percentage of the total area of the breast. The patients were then assigned to one of the four categories of breast parenchymal density distribution established by the Breast Imaging Reporting and Data System (BI-RADS): type 1, the breast is almost entirely fat (glandular parenchyma < 25% of the total area of both breasts); type 2, scattered fibroglandular densities (25%-50%); type 3, heterogeneously dense breast tissue (51%-75%); type 4 extremely dense (> 75% glandular).

It is well known that the sensitivity of mammography is decreased in type 3 and 4 [32, 33] and the patients participating in our study were therefore divided into two groups: dense breast (DB) which included BI-RADS type 3 and 4, and non breast dense (NDB) which included BI-RADS type 1 and 2. In case of contradictory judgments, the classification assigned by at least two readers out of three was considered correct.

Table 1. — *Main characteristics of the patients versus mammographic features: To assess the association between mammographic density and IGF-1, IGFBP-3 and molar ratio, Student's t-test was used. Significant level was $\alpha = 0.05$.*

Variables	Non-dense breast (n = 145; 42.5%)	Dense breast (n = 196; 57.5%)	*p* value (=)
Serum peptide assays			
IGF-1 (ng/ml; mean)	96.6 ± 35.0	109.6 ± 36.1	0.001
IGFBP-3 (ng/ml; mean)	3.8 ± 1.0	3.8 ± 0.8	NS
Molar ratio (mean)	25.5 ± 7.6	29.4 ± 8.6	0.001

NS = not significant.

Table 2. — *Association between plasma level of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio and breast density in premenopausal and postmenopausal women.*

Variables	Premenopausal (n = 150; 43.9%)			Postmenopausal (n = 191; 56.1%)		
	NDB (n = 26; 17.3%)	DB (n = 124; 82.7%)	*p* value	NDB (n = 119; 63.4%)	DB (n = 72; 36.6%)	*p* value
IGF-1 (ng/ml)	107.9 ± 39.3	115.7 ± 36.2	NS	96.6 ± 44.3	98.9 ± 33.5	NS
IGFBP-3 (ng/ml)	3.8 ± 0.9	3.7 ± 0.7	NS	3.8 ± 1	3.7 ± 0.9	NS
IGF-1/ IGFBP-3 Molar ratio	28.7 ± 8.7	31.0 ± 9	NS	29.9 ± 9	26.5 ± 7.1	NS

NS = not significant.

Peptide assays

At recruitment, a peripheral venous blood sample was drawn for determination of IGF-1 and IGFBP-3 levels.

All blood samples were drawn between 8 am and 11 am after an overnight fast; in women of childbearing age samples were drawn between the 6th and 10th day of the menstrual cycle. Serum obtained by centrifuging the blood samples was immediately frozen at -25°C until analysis which was performed in a single block. Blood samples were analysed by a laboratory technician who was blinded to the parenchymal group (DB or NDB) assigned to the patients. A serum sample of each patient was stored for possible later tests. Determination of IGF-1 and IGFBP-3 levels was performed using Immulite 2000 (Siemens Medical Solutions Diagnostics) based on automated sandwich chemiluminescence immunoassay. Values were determined and calibration was performed on a laboratory instrument according to the producer's instructions. IGF-1/IGFBP-3 molar ratio was then calculated.

Statistical analysis

To assess whether classification of DB and NDB was consistent, agreement between the three readers was evaluated using Cohen's Kappa before further statistical analysis.

To assess the association between mammographic density and IGF-1, IGFBP-3 and molar ratio before and after stratified by menopausal status, the Student's t-test was employed. Significant level was set at $\alpha = 0.05$. If the *p* value was > 0.05 the result was reported as not significant (NS).

Results

A total of 7,000 women were assessed for eligibility; 3,099 were excluded because they did not meet the inclusion criteria and 3,560 were excluded according to exclusion criteria. This selection produced a final sample of 341 women. Sampling strategy is illustrated in Figure 1.

Evaluation of mammographic features showed the presence in the sample of 196 (57.5%) patients with DB (BI-RADS 3 and 4) and 145 (42.5%) patients with NDB (BI-RADS 1 and 2). Assessment of inter-operator variability did not show statistically significant differences; Cohen's Kappa values ranged from 0.85 to 0.89 ($p = 0.001$) thus indicating a high level of agreement.

Analysis of the relationship between mammographic density and plasma levels of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio showed that IGF-1 levels and molar ratio varied in the two groups resulting in higher mean values in the DB group (IGF-1: 109.6 vs 96.6 ng/ml; $p = 0.001$ and molar ratio 29.4 vs 25.5 ng/ml; $p = 0.001$) whereas IGFBP-3 showed similar values in the two groups (Table 1).

As regards menopausal status, 150 (43.9%) women were premenopausal and 191 (56.1%) were postmenopausal at the time of mammography. Mean age was 43.9 years for premenopausal women and 61 years for postmenopausal women.

Comparing the association between plasma level of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio and breast density after stratification of the study population by menopausal status (premenopausal and postmenopausal), it was observed that there was no association either in premenopausal or in postmenopausal patients (Table 2).

Discussion and Conclusion

There is an increasing interest in early detection of risk factors for developing breast cancer. Mammographic density is one factor [20-24] but the IGF system has recently been shown to have a role in the development of breast cancer [2, 5-7, 12-14]. However, it is not yet clear whether these factors are interrelated and if and how they are influenced by menopausal status [24-31].

The purpose of this cross-sectional study was to examine the possible effects of IGF-1, IGFBP-3 and IGF-1/ IGFBP-3 molar ratio on mammographic density and assess whether this relationship was similar in subgroups of pre-and postmenopausal women.

The study sample was fairly homogeneous as only Italian Caucasian women were enrolled, while women of different ethnic origins were excluded due to the possibility that plasma levels of IGF-1 and IGFBP-3 and parenchymal density might vary among different ethnicities. This choice was dictated by the need to build a homogeneous study sample, as previous studies of IGF-1 and IGFBP-3 reported in the literature seem not to have paid attention to ethnic differences but only to geographic location thereby suggesting an environmental rather than genetic influence, whereas parenchymal density is thought to differ according to ethnicity rather than geographical location [27, 34, 35]. Also women who had received HRT for up to 12 months before recruitment

were excluded from this study because the use of postmenopausal hormones has been reported to lower circulating IGF-1 levels and increase breast density [31, 36].

Particular attention was paid to the uniformity of blood sampling for determining IGF-1 and IGFBP-3 levels. Analysis of a single sample was considered sufficient, as most authors claim that one evaluation can predict long-term levels of these peptides [37-39]. In premenopausal women, the blood sample was drawn between 8 am and 11 am after an overnight fast between the 6th and 10th day of the menstrual cycle as these values may vary according to the menstrual cycle [40]. Blood analysis was carried out in a single block by one single laboratory technician who was blinded to the parenchymal classification. Using this strategy, peptide levels measured in our group of Italian women were generally lower than those reported by other authors [41].

This study has some limitations. One concerns the reliability of mammographic classification which was performed qualitatively and not by a computer-assisted method. However, BI-RADS mammographic classification is the most common technique used in the USA for the assessment of mammographic density [32]. In order to further reduce the risk of measurement error, women were enrolled only if they had undergone mammographic examination at our center not more than three months before recruitment and blood sampling. Rigid criteria were furthermore used for assessing breast density [42]. Using this method, inter-operator variances were not statistically significant as there was a high level of concordance in the evaluation carried out by the three blinded readers. Furthermore, the BI-RADS system was developed to alert the referring clinician that the ability to detect small cancers in the dense breast is reduced and it not related to the risk *per se* [33].

A second limitation is that the temporality of the relation between growth factors and breast density cannot be determined due to the cross-sectional design. Finally an analysis of the potential confounders of the relationship between mammographic density and plasma level of IGF-1, IGFBP-3 and molar ratio was not carried out.

Our results showed that IGF-1 values and molar ratio were higher in the DB group compared to the NDB group. IGFBP-3 values were similar in the two groups. When the levels of growth factors were compared to breast density stratifying by menopausal status, no association was found.

Previous studies showed that breast cancer risk rose steadily with increased percentage of the breast area with a dense appearance on a prediagnostic mammogram and this association was not explained by other breast cancer risk factors [21-24, 43]. It is still not known through what mechanism breast density is related to cancer risk [23, 44].

On the other hand current breast density reflecting the proportion of stromal and epithelial proliferation may simply indicate the area of susceptible tissue (number of epithelial cells) or may represent the interaction between stromal and epithelial proliferation influenced by local growth factors, including IGF-1 [45]. Growing evidence indicates that breast development and involution are influenced by IGFs [which increase proliferation) and IGFBPs (which reduce proliferation] [46]. Thus, greater breast density may be a consequence of higher IGF and molar ratio levels and an associated increase in proliferation and/or of decreased IGFBP levels with a resulting reduction in the involution process.

Our study provides strong evidence of a crude association between breast density and plasma levels of IGF-1 and molar ratio, but unlike previous studies by other authors, they do not confirm that IGF-1 can be considered determinant in breast density either in premenopausal [13, 15, 24-28] or in postmenopausal women [29-31].

In conclusion, on the basis of our results it is reasonable to assume that the role of IGF-1 and molar ratio in the pathogenesis of breast cancer is mediated through mammographic density. Thus IGF-1 and molar ratio might increase the risk of cancer by increasing the mammographic density.

Further studies are required to clarify these issues, particularly the mechanisms regulating the IGF bioavailability in the biological systems which may explain the development of not only breast cancer, but also prostate, colon and lung cancer in which growth factors have been implicated.

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