Aim of the study: To assess serum levels of ANP in breast cancer female patients and its relationship to metastasis and some clinical parameters among those patients.

Material and methods: One hundred breast cancer patients with and without metastasis along with 20 healthy closely matched controls, were enrolled in the present cross sectional study. Background: To assess the serum levels of atrial natriuretic peptide in breast cancer Serum levels of ANP were assessed using ELISA.

Results: Mean serum levels of ANP breast cancer patients (13.9 ±10.1 ng/ml) were significantly elevated compared to healthy control group (2.2 ±1.3 ng/ml) (p < 0.001). The metastatic breast cancer patients showed significant elevated ANP levels (17.1 ±8.9 ng/ml) compared to non-metastatic group (6.4 ±8.8 ng/ml) p < 0.001. Within the metastatic group significant difference was detected between de novo metastatic, under follow-up, under hormonal control and locally advanced group (p = 0.007).

Conclusions: This study showed significant elevated levels of ANP in the serum of metastatic breast cancer patients compared to non-metastatic patients. Within the metastatic group the lowest levels were detected in metastatic breast cancer under hormonal treatment either tamoxifen or aromatase inhibitor.

Key words: atrial natriuretic peptide, breast cancer, metastases.
Serum atrial natriuretic peptide: a suspected biomarker of breast cancer

They were selected from patients admitted to the Oncology Centre, Mansoura University from December 2014 to November 2015, one day every week. A complete history and clinical examination with special attention to signs and symptoms related to heart failure were performed. Routine laboratory investigations and ANP were also done.

Exclusion criteria
1. Patients with left ventricular dysfunction or coronary artery disease.

Table 1. Patient characteristics

| Parameter                              | No. of patients (100) | %  |
|----------------------------------------|-----------------------|----|
| WHO performance status                 |                       |    |
| 0                                      | 20                    | 20 |
| Fully active, able to carry on all pre-disease performance without restriction | 29 | 29 |
| 1                                      | 51                    | 51 |
| Restricted in physically strenuous activity but ambulatory | | |
| 2                                      |                       |    |
| Ambulatory and capable of all self-care but unable to carry out any work activities | | |
| Primary tumours                         |                       |    |
| Ductal adenocarcinoma (invasive and/or in situ) | 58 | 58 |
| Lobular adenocarcinoma (invasive and/or in situ) | 30 | 30 |
| Paget’s disease (with or without invasive ductal or intraductal component) | 12 | 12 |
| Stage (TNM):                           |                       |    |
| I                                      | 12                    | 12 |
| II                                     | 18                    | 18 |
| III                                    | 15                    | 15 |
| IV                                     | 55                    | 55 |
| HER2 receptor                          |                       |    |
| HER2 (+)                                | 48                    | 48 |
| HER2 (-)                                | 52                    | 52 |
| ER status                              |                       |    |
| ER (+)                                  | 81                    | 81 |
| ER (-)                                  | 19                    | 19 |
| PR status                              |                       |    |
| PR (+)                                  | 82                    | 82 |
| PR (-)                                  | 18                    | 18 |
| Systemic treatment                     |                       |    |
| surgery (modified radical mastectomy)  | 35                    | 35 |
| radiotherapy                           | 30                    | 30 |
| chemotherapy                           | 35                    | 35 |
| endocrine therapy                      | 20                    | 20 |

Group 2b (n = 20): Metastatic breast cancer de-novo (i.e. new breast cancer cases are initially stage 4 or metastatic).

Group 2c (n = 20): Metastatic breast cancer under hormonal treatment with either tamoxifen or aromatase inhibitors and (oestrogen receptor) ER and/or (progesterone receptor) PR receptor are positive.

Group 2d (n = 15): Metastatic breast cancer under follow-up with ER and/or PR receptor are negative.

Group 2e (n = 15): Locally advanced breast cancer is invasive breast cancer that has not received chemotherapy and has one or more of the following features:
• may be large (typically bigger than 5 cm),
• may have spread to several lymph nodes in the axilla or other areas near the breast,
• may have spread to other tissues around the breast such as skin, muscle, or ribs.

They were selected from patients admitted to the Oncology Centre, Mansoura University from December 2014 to November 2015, one day every week.

A complete history and clinical examination with special attention to signs and symptoms related to heart failure were performed. Routine laboratory investigations and ANP were also done.

Informed consent was obtained from all participants prior to their enrolment in the study, and approval from the Local Ethics Committee of Mansoura University was also obtained with reference cod R/17.03.29.
2. Patients who received adjuvant anthracycline-based chemotherapy.
3. Chest wall irradiation.

Sample collection

Three millilitres of venous blood was withdrawn after 12–14 hours of overnight fasting. The blood samples were collected via clean venipuncture and were delivered into plain vacutainer tubes, left to clot for 20 minutes at 37°C, and then centrifuged at 3000 g for 10 minutes. The separated serum was further divided into two aliquots. The aliquots were kept at –70°C for ANP assessment.

Biochemical analyses

Serum ANP levels were detected by enzyme-linked immunosorbent assay (ELISA) technique using kits supplied by ELAab (catalogue no. E0225h) with range 0.156–10.0 ng/ml [7].

Statistical analysis

The statistical analysis of data was done by using SPSS program (statistical package for social science) version 20. The quantitative data were expressed as range and mean ± standard deviation (SD), while qualitative data were expressed in number and per cent. For quantitative data Student’s t-test was used for the comparison between two groups while one way ANOVA test was used to compare among the groups. For qualitative data, the \( \chi^2 \) test was used to compare among the groups. Statistical significant difference was considered at \( p < 0.05 \), and highly significant difference at \( p < 0.001 \).

Results

Mean serum levels of ANP were significantly elevated in breast cancer patient groups (13.9 ±10.1 ng/ml) compared to controls (2.2 ±1.3 ng/ml) \( p < 0.001 \) (Table 2).

Mean serum ANP levels were significantly increased in metastatic breast cancer patients (17.1 ±8.9 ng/ml) compared to non-metastatic breast cancer patients (6.4 ±8.8 ng/ml) \( p < 0.001 \). A non-significant difference was detected in ER%, PR%, and HER2 when compared metastatic to non-metastatic patients (Table 3, Fig. 1).

One-way ANOVA within the four groups of metastatic breast cancer patients using the serum ANP as the dependent variable revealed that there were significant differences in ANP levels between groups \( (p = 0.007) \) (Table 4, Fig. 2).

No association was detected between serum ANP levels and ER, PR, HER2, and breast cancer stage (Table 5).

The ROC analysis to assess the sensitivity of ANP revealed the ability of ANP to discriminate between the control and breast cancer patients and between metastatic and non-metastatic breast cancer patients. The area under curve (AUC) was 0.791 and 0.808, respectively. By using a cutoff value of 4.75 ng/ml between control and breast cancer patients the sensitivity was 69.4 and specificity was 100 (Table 6 and Fig. 3). As regards metastatic and non-metastatic breast cancer patients the cutoff value was (11.4 ng/ml) and the sensitivity and specificity were 78.6 and 78.6, respectively (Table 6).

Discussion

Breast cancer is the most common cancer and the leading cause of cancer death in women worldwide [8]. The development of breast cancer starts with ductal hyperproliferation, followed by subsequent evolution to carcinoma in situ, invasive carcinoma, and finally into metastatic disease [9]. Besides the role of ANP in cardiovascular homeostasis, it has the ability to inhibit tumor growth both in vitro and in vivo [10].

This study reveals significantly higher ANP levels in breast cancer patients (metastatic and non-metastatic) compared with controls \( (p < 0.001) \) (Table 2). This is in agreement with Vesely et al., who reported that breast adenocarcinomas growing in vivo have receptors that mediate ANP’s effects. After binding of ANP to their receptors, the anticancer mechanism of action begins [11].

Table 2. Comparison of the age and ANP levels between patients and control groups

|                  | Patients (n = 100) | Controls (n = 20) | t test | p    |
|------------------|-------------------|------------------|--------|------|
| Age (years)      | 45.9 ±11.3        | 41 ±10.8         | 1.816  | 0.072|
| ANP (ng/ml)      | 13.9 ±10.1        | 2.2 ±1.3         | 5.131  | < 0.001|

Table 3. Comparison of the age and ANP levels between non-metastatic and metastatic breast cancer patients

|                  | Non-metastatic breast cancer patients (group 2a) (n = 30) | Metastatic breast cancer patients (group 2b, 2c, 2d, 2e) (n = 70) | t test | p |
|------------------|------------------------------------------------------|---------------------------------------------------------------|--------|---|
| Age (years)      | 48.9 ±10.2                                           | 45.8 ±11.1                                                  | 1.310  | 0.193|
| ANP (ng/ml)      | 6.4 ±8.8                                             | 17.1 ±8.9                                                   | 5.504  | < 0.001|
| OR, n (%)        | 27 (90%)                                             | 54 (77.1%)                                                 | 2.256* | 0.133|
| PR, n (%)        | 27 (90%)                                             | 55 (78.6%)                                                 | 1.858* | 0.173|
| HER2, n (%)      | 13 (43.3%)                                           | 35 (50%)                                                   | 0.374* | 0.541|

\( \chi^2 \) test

OR – oestrogen receptor; PR – progesterone receptor; ANP – atrial natriuretic peptide
The molecular mechanism underlying the anticancer and anti-proliferative effect of ANP has been mainly related to its interaction with the specific natriuretic peptide receptors (NPRs) and inhibition of some metabolic targets critical for cancer development, including the Ras-MEK1/2, ERK1/2 kinase cascade [12, 13], Wnt pathway [14, 15], VEGF, and B-catenin [16]. DNA synthesis is also inhibited within

Table 4. Comparison in ANP levels between different groups of metastatic breast cancer

|                        | De novo metastatic (n = 20) | Metastatic under hormonal therapy (n = 20) | Metastatic under follow up (n = 15) | Locally advanced (n = 15) | ANOVA test |
|------------------------|-----------------------------|-------------------------------------------|----------------------------------|-------------------------|------------|
| Age                    | Mean ± SD                   | Mean ± SD                                 | Mean ± SD                        | Mean ± SD               | F          |
| Age                    | 41.6 ±10.4                  | 44.6 ±12                                  | 46.6 ±11.6                       | 42.9 ±10.8              | 0.642      |
| ANP (ng/ml)            | 18.1 ±4.5                   | 11.2 ±10.2                                | 15.8 ±11.7                       | 21.3 ±6.5               | 4.410      |
| OR, n (%)              | 20 (100%)                   | 20 (100%)                                 | 0 (0%)                           | 14 (93.3%)              | 64.707*    |
| PR, n (%)              | 20 (100%)                   | 20 (100%)                                 | 0 (0%)                           | 15 (100%)               | 70.000*    |
| HER2, n (%)            | 15 (75%)                    | 12 (60%)                                  | 0 (0%)                           | 8 (53.3%)               | 20.867*    |

* χ² test
OR – oestrogen receptor; PR – progesteron receptor; ANP – atrial natriuretic peptide

Table 5. The association of OR, PR, HER2, and tumour stage with ANP in the patients with breast cancer

|                          | ANP (ng/ml) t test           |
|--------------------------|------------------------------|
|                          | Mean ± SD                  | t     | p       |
| OR                       |                             |
| Absent                   | 13.8 ±11.7                  | 0.014 | 0.989   |
| Present                  | 13.9 ±9.8                   |       |         |
| PR                       |                             |
| Absent                   | 16.3 ±12.2                  | 1.134 | 0.260   |
| Present                  | 13.3 ±9.6                   |       |         |
| HER2                     |                             |
| Absent                   | 13.5 ±10.4                  | 0.346 | 0.730   |
| Present                  | 14.2 ±9.9                   |       |         |
| Stage of tumour          |                             |
| I                        | 13.5 ±17.8                  | 2.601*| 0.079   |
| II                       | 11.3 ±11                    |       |         |
| III                      | 15.9 ±8.8                   |       |         |

F value, ANOVA test
ANP – atrial natriuretic peptide; OR – oestrogen receptor; PR – progesteron receptor

The molecular mechanism underlying the anticancer and anti-proliferative effect of ANP has been mainly related to its interaction with the specific natriuretic peptide receptors (NPRs) and inhibition of some metabolic targets critical for cancer development, including the Ras-MEK1/2, ERK1/2 kinase cascade [12, 13], Wnt pathway [14, 15], VEGF, and B-catenin [16]. DNA synthesis is also inhibited within

Fig. 1. Comparison of the ANP between non-metastatic and metastatic breast cancer patients

Fig. 2. ROC curve of ANP for discrimination between cases and controls

Fig. 3. ROC curve of ANP for discrimination between metastatic and non-metastatic BC
The present study also showed that within the metastatic patient groups, circulating ANP levels was lowest in metastatic patients who received hormonal therapy either tamoxifen or aromatase inhibitors. This was in agreement with the study by Silva et al. [25], who found decreased levels of NT-ProBNP in patients receiving tamoxifen, and attributed this to the role of tamoxifen in preventing sub-clinical cardiac damage and decreasing cardiac synthesis of pro-BNP through different mechanisms. The first is the stimulation of endothelial nitric oxide synthase (eNOS) activity and promotion of antioxidant effects by increasing catalase activity [26]. The second was through the promotion of a significant increase in the antioxidant activity of glutathione and glutathione peroxidase [27]. As regard to the ROC analysis to assess the sensitivity of ANP in the discrimination between the control and breast cancer patients and between metastatic and non-metastatic breast cancer patients. By using a cut off value of 4.75 ng/ml between control and breast cancer patients the sensitivity was 69.4 and specificity was 100 (Table 6, Fig. 3). As regards metastatic and non-metastatic breast cancer patients the cutoff value was 11.4 ng/ml, and the sensitivity and specificity were 78.6 and 78.6, respectively (Table 6).

Points of strength: to our knowledge the current study is the first report showing the diagnostic value of ANP in breast cancer and its relationship with metastasis and some clinical parameters.

One limitation of our study is the limited number of participants. The disparity between our results and some reported studies could be due to sample size limitation, different ethnic groups, and different environmental factors. Therefore, additional high-quality research to consider this peptide as a biomarker for assessing detection, progression, and early intervention therapy strategies in breast cancer patients with large sample sizes should be carried out to verify the association.

From this study, we can conclude that ANP, a cardiovascular hormone used as a targeted therapy for heart failure, may be a suspected marker for distant metastases in breast cancer patients.

The authors declare no conflict of interest.

Table 6. AUC and performance characteristics of ANP for discrimination between cases and controls, as well as between non-metastatic and metastatic

| Discrimination between | Cut off | AUC   | p       | 95% CI    | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Accuracy (%) |
|------------------------|---------|-------|---------|-----------|----------------|----------------|---------|---------|--------------|
| Cases and control      | 4.75    | 0.791 | < 0.001 | 0.713-0.869 | 69.4           | 100            | 100     | 40      | 74.6         |
| Non-metastatic and metastatic | 11.4    | 0.808 | < 0.001 | 0.704-0.912 | 78.6           | 78.6           | 90.2    | 59.5    | 78.6         |

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