The Intrasubject Variability in the Acute Phase Protein (Orosomucoid) Levels and their Influenced by Breast Malignancy

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Abstract

Background: The major healthcare burden is breast cancer, screening services are expensive and difficult to organize, requiring major issues of administrative and quality control. however a potential biomarker that could help in the screening of the disease progress might improve the available routine mode of diagnosis. This study was aimed to examine the variability of Orosomucoid levels in breast cancer patients also to review the background documents on the state of the art of the scientific literature in this area of work. **Materials and Methods:** A cross sectional study was conduct on a breast cancer patients which were collected from oncology unit , Al-Hussein teaching Hospital in Kerbala / Iraq. Enzyme Linked Immunosorbent Assay system (ELISA) was performed using Sandwich method to measure the concentrations of serum Orosomucoid protein levels. **Results:** The primary stage of breast cancer was shown a wide spread range of the protein levels (95.4- 664.5 ng/ml) compared to the metastasis stage range (59.0- 376.3 ng/ml). The effect of disease duration and drug therapy also examine. Long term of duration of the disease with chemotherapy and/or hormonal therapy might decrease the level of protein, but, no significant differences were found. **Conclusion:** Since rosomucoid protein works as transporter, the protein may be used as an indication of the drug response due to decreased level in patients who were taking both chemotherapy and hormone therapy. Determination of serum rosomucoid protein could guide treating oncologist to have an idea to what extent the patients have response.

Keywords: *intrasubject variability; acute phase protein; Orosomucoid; breast Malignancy*

Introduction

Human plasma alpha-1-acid glycoprotein (AGP, also known as orosomucoid) is an acidic (pKa = 2.6) glycoprotein that is highly soluble in water . AGP is one of the most heavily glycosylated proteins in human plasma, and approximately 45% of its molecular weight (41–43 kDa) is composed of glycosylations. It has been suggested to have anti-inflammatory or immunomodulatory activity, although its role in plasma is not clear .Changes in the level of AGP in the blood are associated with systemic tissue injury, infection and inflammatory responses, and with an increase in hepatic AGP synthesis. Therefore, it has been posited that expression of AGP affects mainly interleukin-1β (IL-1β), tumor necrosis factor-α (TNFα), interleukin-6 and IL-6-related cytokines .Serum AGP levels increase in various types of cancer. They are higher in hepatocellular carcinoma than in chronic liver disease ,and are elevated in patients with gastric cancer compared to healthy volunteers .There seems also to be an important link between ovarian cancer and elevated levels of AGP ,and it has been proposed that a decrease in AGP level is associated with remission of lung cancer, and an increase in AGP level with progression[1;2;3;4].

Although the concentration of AGP alone is not diagnostic for a particular pathological condition, the altered glycosylation of AGP (microheterogeneity) in different diseases, provides an alternative biomarker

target. These alterations have the potential to be markers for particular diseases and also disease progression[5]. In most disease states including inflammation, infection, and cancer, AAG levels increase from 2 to 6-fold in humans ,and show a much broader fold of induction in animals from 2 to 20-fold depending on animal species and disease. While the biological role of AAG remains unclear, it has been demonstrated to regulate immunity and play a role in both pro- and anti-inflammatory response. AAG has long been used as a clinical biomarker, and the potential to expand its application for disease diagnosis, prognosis, and characterization has grown given the recent advances in proteomics and high resolution mass spectrometry[6;7].

Synthesis of ORM

ORM is predominantly synthesized by hepatocytes and parenchymal cells, upon stimulation by proinflammatory cytokines. It is then released into the blood and distributed in body fluids, including plasma, mucus, gastric juice, and jejunal fluid. About 60 % of ORM in the body is present in the central compartment and the remainder in a peripheral compartment, most likely the extravascular space. The plasma concentration of ORM is increased in response to various stressful, physical trauma, bacterial infection, and unspecific inflammatory. The levels of liver AGP mRNA and plasma ORM protein increase 10- to 200-fold within 24 h of experimentally induced inflammation in rats, mice, and rabbits. Human breast epithelial cells, type II alveolar epithelial cells, human microvascular endothelial cells, human granulocytes, the monoblastoid cell line THP-1, monocytes, macrophages, polymorphonuclear leukocytes, and granulocytes have been shown to synthesize and secrete ORM. Constitutive ORM gene expression has been observed in extrahepatic organs such as lung, breast, kidney, and adipose tissue. Indeed, there is a growing body of evidence that ORM1 could be secreted by extrahepatic tissue during various pathological states. Croce et al. showed by an immunohistochemistry technique that ORM was localized in isolated colorectal carcinoma samples[8;9;10].

Acting as an acute-phase reactant and disease marker

Systemic injury induces a drastic change in the hepatic production of many plasma proteins, namely the acute phase reactants. Acting as a positive acute-phase reactant during acute-phase response, ORM concentration can elevate 1–10 times during several pathological conditions depending on the severity of the disease state and the various stimulating factors including infection, inflammation, tumor, surgery, tissue injury, sepsis, and necrosis. Although the pathophysiological mechanisms responsible for this markedly increased excretion are unknown, monitoring of ORM excretion may provide a window for clinically relevant observation of changes in various disease processes. Therefore, the level of ORM in body fluid appears to be a biological marker in clinical practice. glycosylation of ORM suggests it is a strong candidate as a marker of the progression and prognosis of various cancers, with various glycoforms containing highly fucosylated tri- and tetra-antennary oligosaccharide side chains, which typically indicate a poor prognosis [11,12].

Materials and Methods

The present work included a cross sectional study for a group of 40 patients with different stage of breast cancer which were selected from oncology unit, Al Hussein Medical City. The protocol of the study was approved by Ethical Committee of Kerbala Medical College, and committee of oncology unit in Al Hussein Teaching Medical City. The sociodemographic aspects of the patients were collected through the self-reported technique (questionnaire) including age, history of family, BMI, stage and grading, duration of disease, having Chemotherapy and/ or hormonal therapy. Enzyme Linked Immunosorbent Assay system (ELISA) was performed using Sandwich method to measure the concentrations of serum Human α1-Acid glycoprotein following the assay procedure.

Results

Concentration of AAG were measured in (40) female breast cancer patients. The clinical demographic characteristics and laboratory parameters of patients were summarized in Table 1 The Table illustrated the mean age of participants which was within the age group of (30– 72) years old. Females were divided into sub-groups such as ages groups, different cancer stages to primary and metastatis, patients having only chemotherapy and/or Hormonal therapy, duration of disease, and BMI.

Table 1 clinical characteristics of study participants

Age (year): Mean (range)	51 (30– 72)
BMI	32
Duration of disease (month): Mean (range)	32 (2-84)
Primary cancer/ Metastatis: n(%)	70% / 30%
Patients having chemotherapy: n (%)	21(53%)
Patients having chemotherapy+ hormonal therapy: n (%)	19 (47%)

Examination the distribution of data

A box plot was used to visually showing the distribution of data through displaying the data quartiles (or percentiles) and averages. Box plots show the five-number summary of a set of data: including the minimum score, first (lower) quartile, median, third (upper) quartile, and maximum score. Figure 1 was demonstrated the distribution of serum AAG levels in breast cancer patients based on primary and metastasis stages.

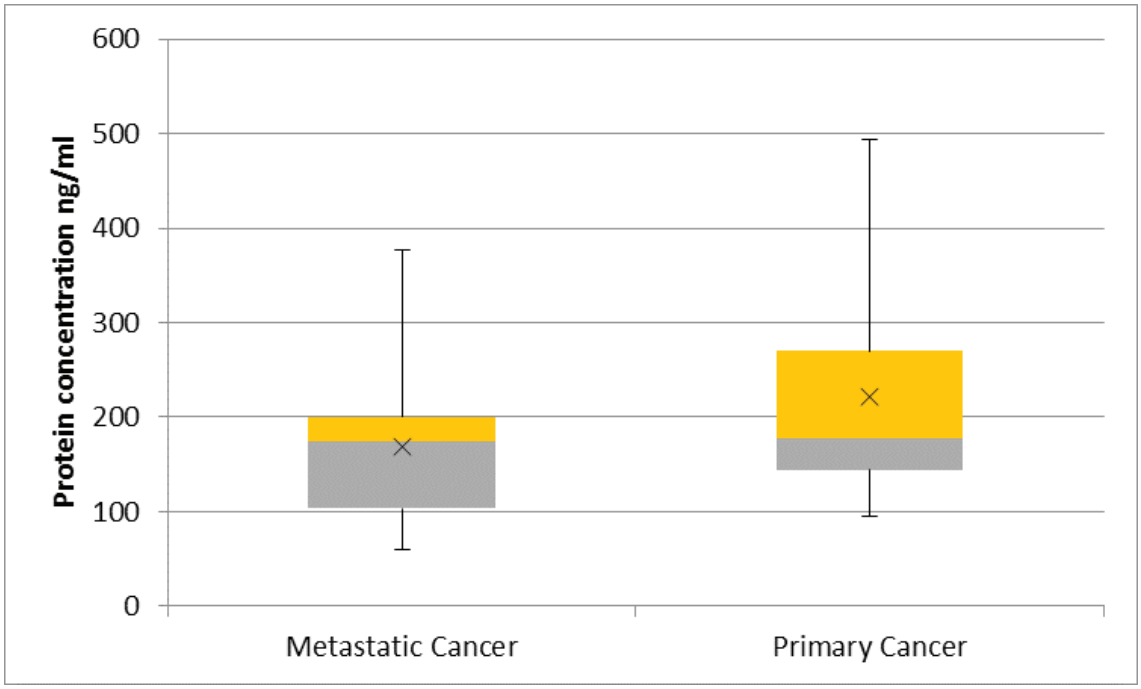

Figure 1 Distribution of serum AAG levels in Primary and Metastatic breast cancer patients.

The primary stage was shown a wide spread range of the protein levels (95.4- 664.5) compared to the metastasis stage range (59.0- 376.3), no significant difference was found between the two groups p value > 0.05. That difference could result from changes in the expression of the genetic variants of AAG and that might be due to the observed AAG phenotypes. Previous studies showed a three main phenotypes of AAG (ORM1 F1, ORM1 S, and ORM2 A) , the clinical stage of the cancer disease may affect AAG levels and its variants[13].

. Moreover, the elevated expression of AGP1 was significantly correlated with large tumour size, advanced TNM stage and positive distant metastasis. The results suggested that AGP might be an oncogene promotes the malignant tumour progression , and It might be correlated with aggressive clinical characteristics[14].

Furthermore, the distribution of serum AAG levels were examined related to the BMI of the patients. There were a clear decrease in the protein level by comparing different BMI groups (grouping was performed using the Sturgess formula[15].) as shown in figure 2. The nonparametric test (Spearman rank test) (Coefficient r_s) was used for the analysis of the difference in quantitative data between the groups. Results were indicated a significant negative correlation between BMI and the protein levels (r_s = - 0.53, p=0.0005) in breast cancer patients.

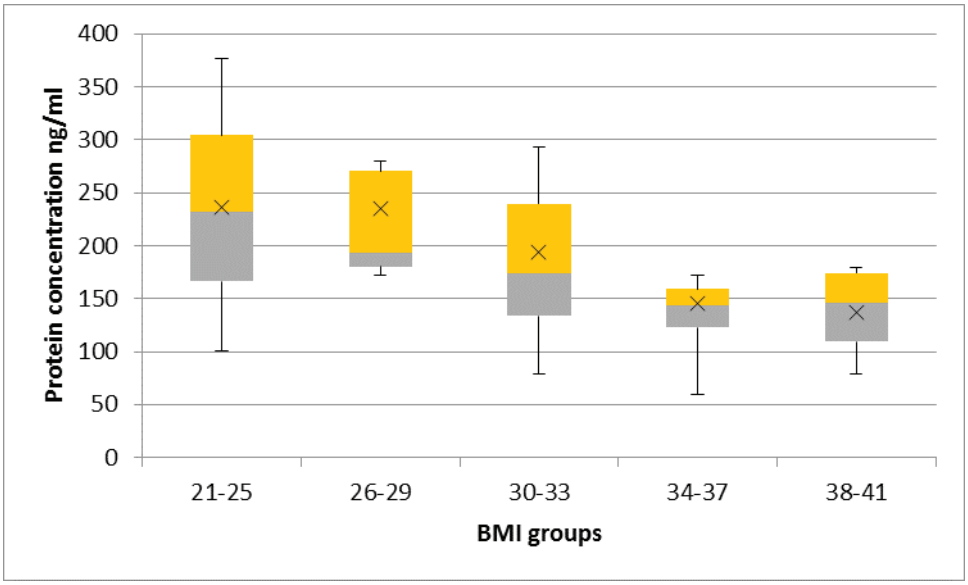

Figure 2: Distribution of serum AAG levels in breast cancer among different body mass index groups.

Generally, Obesity is associated with increased breast cancer risk[16]. but no previous study was indicated any direct mechanism for the association between obesity in breast cancer women and serum AAG levels. Maachi et al. reported only relationship between the level of adipose tissue content in the acute phase proteins. Their results illustrated a significant correlation between AAG and the adipose tissue content namely in IL-6 and α-TNF, However, no significant correlations were found between AAG and circulating levels of IL-6, αTNF[17]. That might reflect an indirect relationship between these cytokines which could be secreted by adipocytes and by inflammatory cells such as macrophages present in adipose tissue from obese subjects [18]. also it might be related to increased fat amount not the body mass. This could explain the AAG levels observed in obese patients[19]. which deleterious since it has been found to promote directly endothelial cell inflammation processes [20].

On the other hand, The effect of duration of disease and chemotherapy with/without Hormonal therapy also examine. Figure 3 & 4 were illustrated the distribution of serum AAG levels based on the duration of disease and patients having chemo and/or hormonal therapy. Increasing the duration of breast cancer was shown a fluctuation in the protein level by comparing different duration groups as shown in Figure 3. There mean of the protein level in patients having chemotherapy and patients having chemotherapy plus hormonal therapy were 196.6, 210.3 ng/ml respectively as shown in figure 4. Difference in the mean protein level according to the duration of disease and cancer therapy were not correlate significantly p value > 0.05.

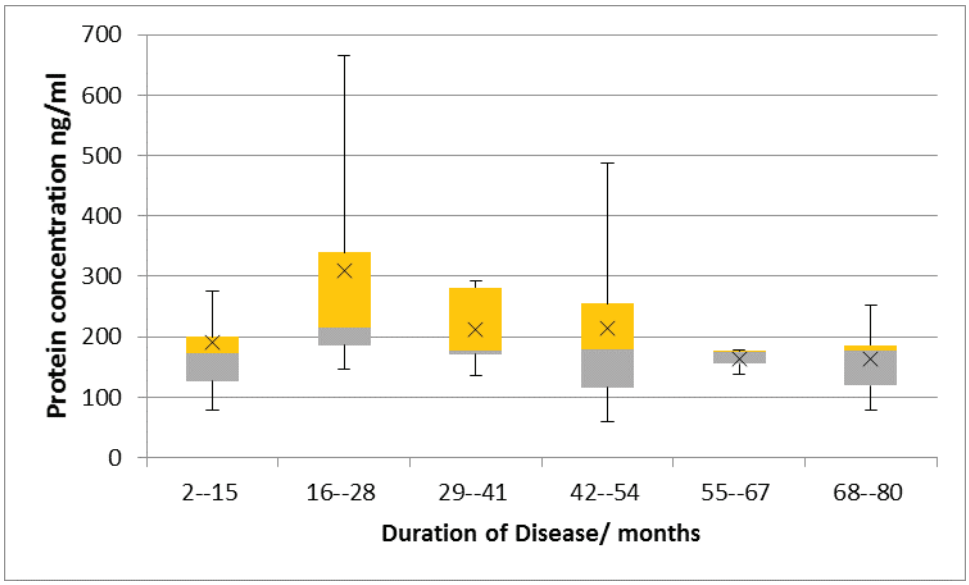

Figure 3: Distribution of serum AAG levels in breast cancer among different duration of breast cancer groups.

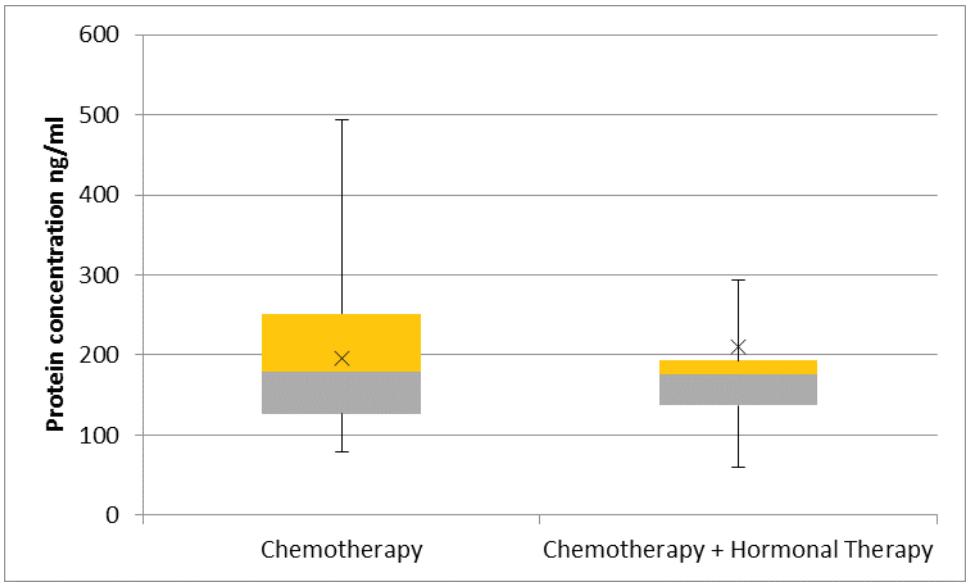

Figure 4: Distribution of serum AAG levels in breast cancer among patients having chemotherapy and/or Hormonal therapy groups.

AAG concentration can elevate 1–10 times during several pathological conditions depending on the severity of the disease state[21]. It also acts as a sensitive index reflecting the level of inflammation and degree of injury to tissues as well as an evaluator of treatment outcome[22].

Although the pathophysiological mechanisms responsible for this markedly increased excretion are unknown, monitoring of ***AAG*** excretion may provide a window for clinically relevant real time observation of changes in various disease processes. Therefore, the level of ***AAG*** appears to be a biological marker in clinical practice. ***AAG*** work as an positive acute-phase reactant and disease marker, Any increasing in the ***AAG*** was probably due to increasing the demand of synthesis in the liver ORM1 which might be due to their role as drug carier . Drug therapy is a factor likely to alter AAG levels. Plasma AAG was shown to increase after treatment[23;24].

Generally, ***AAG*** has the ability to bind and carry numerous basic[25]. and neutral drugs from endogenous and exogenous origin due to its physical-chemical properties (pI =2.8–3.8). The binding capacity of a drug mainly depends on the ***AAG*** protein conformation, ligand polarity, temperature, pH. It has been revealed that the binding of ***AAG*** to a membrane results in a secondary structural change from an original (prevalently β- sheet to α-helix structure), causing its tertiary structure to collapse[56]. This structure seems to be an intermediate between the native state and the denatured state (27). It should be noted that some ligands such as estradiol can be bound to up to seven binding sites[28]. and the binding of progesterone induces at least one secondary structure transformation, including a short α-helix to an antiparallel β-sheet [29]..

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Conflict of Interest: None

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