
Correlation of Serum Concentration of Cystatin C & β -2-microglobulin in Pediatric Malignancy

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Abstract

Background: Malignant disease is the second most frequent cause of death between the ages of 1 and 15 years. Childhood tumors are relatively more malignant, disseminating early and responding poorly to treatment, Beta2-microglobulin, a Major Histo-compatibility (MHC) class I subunit, is found to act as a prototypical oncogenic factor capable of stimulating growth and progression of various cancers. Cysteine proteases are proteolytic enzymes involved in many pathological processes such as tumor invasion and metastatic processes.

Aim: to assess the status of Beta2-microglobulin, cystatin C and the effects of chemotherapy on these markers in patients with various malignant diseases.

Materials and methods: the present study is a case-control study done at Al-Kadhimiya Teaching Hospital in 2010. Includes measurement of serum Beta2-microglobulin and Cystatin C in 60 patients with different malignant conditions who were divided into two groups:

- Patients with newly diagnosed malignant tumor **G1**: (n=30).
- Patients with definitely diagnosed malignant tumor **G2**: (n=30), The results were compared with another 30 malignancy-free children who were included as disease-unrelated controls:
- Patients complaining from diseases other than malignancy (**G3**): (n=30).

Results: showed a significant increase in serum Beta2-microglobulin and Cystatin C in patients with malignant tumors as compared with the controls ($p < 0.001$) Moreover, these markers were significantly high in patients on no treatment G1 compared with patients on chemotherapy G2. Additionally, a significant ($P < 0.05$) positive correlation was found between these markers in the studied groups.

Conclusion, pediatric patients with various malignant tumors (but normal renal function) have high level of serum Beta2-microglobulin and cystatin C when compared with controls; for Beta2-microglobulin, this can be explained on the fact that the presence of Beta2-microglobulin on the surface of the numerous tumour cell lines identifies it with the notion of tumour mass; for Cystatin C this can be explained on genetic basis due to the fact that Cys-C gene is one of the most highly up-regulated genes in cancer. The above results were supported by the significant high level of s. Beta2-microglobulin and cystatin C; which can be used as a tumor marker although not specific.

Key words: Beta2-microglobulin, cystatin C, malignancy.

Introduction

Malignant disease is the second most frequent cause of death between the ages of 1 and 15 years. Although malignancy is an important cause of death, it is nevertheless uncommon. Malignancy is not only less common than in adults, it is also different in origin and course. Most childhood malignancy arises from reticuloendothelial system or from nerve or connective tissue. Childhood tumors are relatively more malignant, disseminating early¹.

Beta2-microglobulin (B2M), a MHC class I subunit, is found to act as a prototypical oncogenic factor capable of stimulating growth and progression of various cancers and plays a key regulatory role in stimulating cancer metastasis. Beta2M in serum or urine has been regarded as an independent biomarker in cancers (for both solid tumors and blood malignancies)².

Cysteine proteases are proteolytic enzymes involved in many pathological processes and found in the lysosomes of cells; examples include the cathepsins B, H and L. The role of cysteine proteases is crucial in normal cellular metabolism, being fundamental to intracellular protein turnover, degradation of collagen, and cleaving of precursor proteins³. Currently, cystatin C (CC) is a protease

inhibitor that is involved in processes such as tumor invasion and metastasis, inflammatory processes and some neurological diseases. In such diseases the emphasis is placed on the fine balance and regulation of both the cysteine proteases and their inhibitors, with an imbalance resulting in a pathological state^{3,4}.

This study was conducted to assess the status of (B2M and CC) as a tumor markers and the effects of chemotherapy on these markers CC in patients with various malignant diseases.

Materials & Methods:

A-Patients:

The study was a case-control study conducted on 60 patients with malignancy (solid or hematological) attending the Pediatric Oncology Consultant Clinic at Al-Kadhimiya Teaching Hospital, for evaluation of newly diagnosed malignancy, or for follow up and monitoring chemotherapy during the period from January, 2010 till the end of September, 2010. The sixty patients were divided into two groups (**Table 1**):

1. Patients with newly diagnosed malignant tumor (**G1**): They were 30 patients discovered to suffer from various types of malignant conditions of different stages; with

- normal renal function as indicated by normal serum creatinine; and equal sex distribution.
2. Patients with definitely diagnosed malignant tumor (**G2**): They were 30 patients who suffer from various types of malignant conditions of different stages and on different regimes of chemotherapy; with normal renal function as indicated by normal serum creatinine; and equal sex distribution.
 3. Patients complaining from diseases other than malignancy (**G3**):

The study included another 30 malignancy-free children attending the Pediatric Consultant clinic at Al-Kadhimiya Teaching Hospital, for evaluation of diseases other than malignancy. They were included as disease-unrelated controls matched for age and sex (**Table 1**).

They were 30 patients with normal renal function as indicated by normal serum creatinine; and equal sex distribution. The exclusion criteria used for cases and controls were impaired renal function as it may interfere with the measurement of B2M and CC.

B. Blood samples:

Five milliliters of random venous blood were withdrawn from each patient, in supine position, without application of tourniquet. Samples were transferred into clean new plane tube, centrifuged at 4°C for 15 minutes at 4000×g. and the separated plasma were transferred into a new plane tube where fibrin mesh was removed by a wood stick leaving a free serum which was stored in Eppendorf tube and was used for measurement of cystatin C and creatinine. Samples were processed within 5h after blood collection⁵

C-Methods

Serum B2M levels was determined by ELISA kit (Human beta-2 Microglobulin ELISA Kit from Immundiagnostik) Separation and detection of cystatin C was accomplished by ion pair liquid chromatography and UV detection. Gradient elution mode was utilized to elute Cyst C with a UV detection of 224 nm⁵. Measurement of serum creatinine was done by enzymatic method (Bio Merieux manual, 1986).

D. Statistical analysis:

Statistical analysis was done using Excel system version 2003 and includes descriptive statistics (mean and standard deviation) and inferential statistics (*t*-test) to test the significance of mean difference. When P-value was less than 0.05, the difference is considered statistically significant, and the difference is considered highly significant when P-value was less than 0.001.

Results:

Both *serum B2M* and *serum CC* was significantly elevated in patients with malignancy (G1 & G2) compared with malignancy-free patients (G3) [P < 0.001 for both]. Moreover, serum B2M and serum CC was significantly high in patients on

no treatment (who were newly diagnosed) G1 compared with patients on chemotherapy G2 (who were on various regimes of chemotherapy [P < 0.001] as shown in Table 2.

A significant positive correlation was found between *serum B2M* in patients with malignancy (G1 & G2): G1 (r = 0.9, *P*-value < 0.001) G2 (r = 0.93, *P*-value < 0.001), also this correlation was found in malignancy-free patients (G3) (r = 0.86, *P*-value < 0.001) as seen in Figures 1, 2 and 3.

Discussion:

If the presence of β -2M on the surface of all nucleated cells, as a component of the HLA class I molecule, can be associated with the notion of histocompatibility and thus with the immune response, the presence of β -2m on the surface of the numerous tumour cell lines identifies it with the notion of tumour mass⁶.

Cystatins function as cysteine protease inhibitors, are expressed in numerous cell types, and regulate a number of physiological processes. Four cystatins have been extensively studied: cystatin A, cystatin B, cystatin C, and cystatin M. Aberrant regulation of cystatins occurs in a number of diseases, including cancer and certain neurodegenerative disorders⁷. Recent advances in the understanding of cystatin function suggest that these proteins may regulate promotion or suppression of tumor growth, invasion, and metastasis. Cancer is a disease of abnormal gene expression and cancer cells exhibit aberrant epigenetic events (such as DNA methylation), leading to gene silencing⁸. Cystatins are epigenetically silenced through DNA methylation-dependent mechanisms in several forms of cancer, these findings suggest that DNA methylation-dependent epigenetic mechanisms may play an important role in the loss of cystatin gene expression and protein function during neoplastic transformation and/or tumor progression⁹.

Serum studies in patients with other types of cancer have demonstrated similar results, as levels of Cys C were found to be higher in serum of patients with cancer compared to serum levels of healthy controls as in Zore et al.¹⁰; Miyake et al.¹¹; Mulaomerovic et al.¹² studies. A similar findings concerning B2M were found in other studies like Mink et al.¹³; Asaoku H.¹⁴, Bunning et al.¹⁵

The level of the studied markers (B2M & CC) might be changed also due to pathological renal failures, changing the glomerular filtration rate in cancer patients^{6, 16}. However, patients included in this study, did not exhibit any impaired kidney function as noticed from results of serum creatinine which were in the normal range for age. Therefore, we believe that the results of B2M & CC in this study have not been biased by age-related¹⁷ or pathological changes of kidney function^{6, 16}.

It was observed that almost all patients with cancer had elevated values of CC when compared with controls, while newly-diagnosed patients had higher values of Cys-C even compared to those who

receive chemotherapy. This can be explained by the fact that Cys-C is one of the most highly up-regulated genes in cancer (almost 50-fold up-regulated). The overexpression of Cys-C gene by cancerous cells indicates that its levels in the serum may also reflect tumor burden¹⁸.

Specific antibodies to beta2M have remarkable tumoricidal activity for both solid tumors and blood malignancies and are shown to be selective to tumor

cells, but caused no toxicity in normal cells. These surprising data strongly suggest that B2M is a promising new therapeutic target for human cancers².

Thus, in conclusion, it seems that both B2M & CC reflects both tumor load and renal function in cancer patients and CC levels may be elevated in people with normal GFR reflecting tumor growth.

Table (1): Clinical Criteria of different patient groups (with different malignant diseases or malignancy-free controls).

Group	G1	G2	G3
No	30	30	30
Age / year (Mean + SD)	2.2 $\pm$ 2	4.5 $\pm$ 1.5	4.9 $\pm$ 2
Age range (years)	(0.5-8)	(1-10.5)	(2-10)
BMI / Kg/m ² (Mean + SD)	20.3 $\pm$ 1.7	17 $\pm$ 7.6	20.4 $\pm$ 1.2
BMI range (Kg/m ²)	17.3-23.5	8.7-21.6	7.3-28.5
SA / m ² (mean + SD)	0.9 $\pm$ 0.5	1 $\pm$ 0.3	0.8 $\pm$ 0.3
SA range (m ²)	0.5-1.4	0.9-1.3	0.3-1.4
S. creatinine (μ mol/L) (mean + SD)	49.6 $\pm$ 16.2	60.9 $\pm$ 18.2	57.5 $\pm$ 16.9
S. creatinine range (μ mol/L)	32-76	32-77	34-81

Table (2): The mean serum cystatin C in different pediatric patients groups (presented as mean $\pm$ SD).

Variable	G1 (n=30)	G2 (n=30)	G3 (n=30)
s. B2M (mg/L)	5.8 $\pm$ 2.8*	4 $\pm$ 0.8** ^ψ	1.3 $\pm$ 0.4
s.CC (mmol/L)	4.5 $\pm$ 0.5*	3.5 $\pm$ 0.3**	0.6 $\pm$ 0.2**

(G1): Pediatric patients with diagnosed malignant tumor (no chemotherapy).

(G2): Pediatric patients with definitely diagnosed malignant tumor (on chemotherapy).

(G3): Control pediatric patients complaining from diseases other than malignancy.

*t-test: G1 versus G3, $p < 0.001$, **t-test: G2 versus G3, $p < 0.001$, ^ψ t-test: G2 versus G1, $p < 0.05$

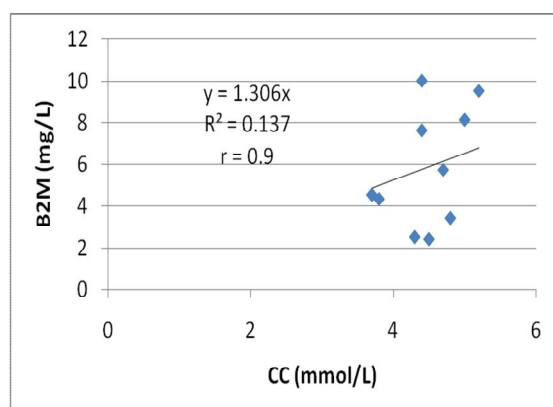


Figure (1): Correlation between serum (B2M & CC) in G1: Patients with malignant diseases on no treatment (who were newly diagnosed) (n=30; $r = 0.9$; $P < 0.001$)

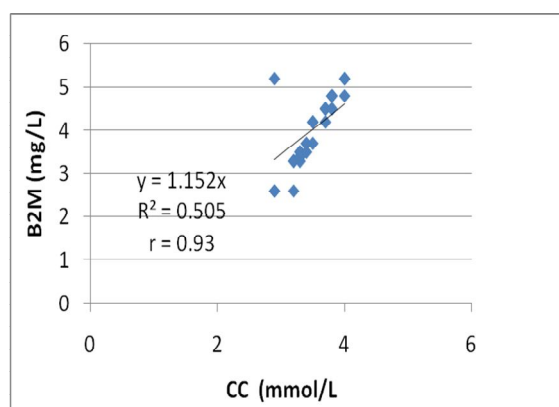


Figure (2): Correlation between serum (B2M & CC) in G2: Patients with malignant diseases on chemotherapy (who were on various regimes of chemotherapy) (n=30; $r = 0.93$; $P < 0.001$)

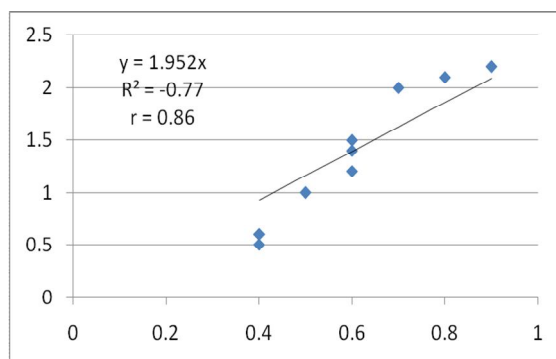


Figure (3): Correlation between serum (B2M & CC) in G3: Patients complaining from diseases other than malignancy (malignancy-free children malignancy-free children (n=30; $r = 0.86$; $P < 0.001$).

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