STUDY OF SERUM LEVELS OF STEM CELL FACTOR IN PATIENTS WITH BLADDER CANCER

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SUMMARY: Stem cell factor (SCF) has recently been identified as a multi-potential growth factor that acts on early different progenitor cells of various lineages and triggers its biologic effects by binding to its receptor, c-Kit. The main aim of this study was to evaluate serum levels of SCF in bladder cancer patients, and its possible relation with clinical course of disease. The serum SCF level was determined in bladder cancer patients, as our subject cases. The study group consisted of 35 bladder cancer patients and 35 healthy individuals as controls. The samples were prepared in two separated times, before operation and 2–3 months after surgery. The achieved data were compared with serum SCF level in healthy controls. In addition, we tested relationship of it with cancer stage and grade as well as with CBC parameters. Serum SCF was measured, using Enzyme Linked Immunosorbent Assay (ELISA) kit. Serum SCF levels in bladder cancer patients were not significantly different from those of healthy controls (p=0.5). Furthermore, in bladder cancer cases, the preoperative and postoperative serum SCF levels were not significantly different (p=0.889). Serum SCF levels were not associated with pathological indicatory, including cancer stage and grade, as well as with CBC parameters. According to the results, it is suggested that serum SCF levels were not significantly different from that in healthy controls, besides serum SCF levels were not associated with cancer stage and grade as well as CBC parameters. In this way, SCF may not be used as a remarkable predictor of bladder cancer recurrence and pathological indicator.

Key Words: Stem cell factor, bladder cancer.

INTRODUCTION

Bladder carcinoma, as the second prevalent urothelial cancer, has a significant importance in human communities. This malignancy includes 7% of all cancers in males and 3% of them in females, in the United States (1). Bladder cancer is the fourth most common cancer in men and the ninth in women (1). Stem cell factor (SCF) acts as a growth factor that triggers its biologic effects by binding to its receptor of c-Kit (2). This growth factor is normally produced by mesenchymal cells such as fibroblasts and endothelial cells (3). SCF regulates the migration (4), and survival of mast cell and promotes proliferation (5) and differentiation of both immature and mature cells (6, 7). Regarding to multi-potential roles of SCF, it could play directly/indirectly many optional activi-

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ties such as: hematopoiesis, angiogenesis (8, 9) and apoptosis (10, 11), and plays predominant roles in growth of processing and differentiation, in a vast range of cells. The SCF receptor is encoded by the c-Kit protooncogene belongs to the transmembrane tyrosine kinas family (12). It is postulated that several growth factors and cytokines such as SCF and TGF are produced in the most stages and grades of numerous malignancies (13). SCF is normally found in both soluble and transmembrane forms. A mutation, which proceeds deletion of the transmembrane and cytoplasmic domains of SCF, results in production of soluble SCF and absence of transmembrane form of SCF (14). This form is constitutively produced by endothelial cells and fibroblasts. Over-expression of SCF mRNA, has been defined in many studies (15,16), regarding to some malignancies such as: neuroblastoma, endometriosis, small cell lung cancer, ovarian cancer, prostate cancer and also is established to express in rhabdomyosarcoma, basal cell carcinoma and even in chronic renal failure and HIV infected patients (14, 17–19).

Mroczko *et al.* (20) had an approach on early diagnoses of non-small cell lung cancer (NSCLC) and used a sensitive sandwich Enzyme Linked Immunosorbent Assay (ELISA) system to assay the SCF serum levels in 34 cases of NSC lung cancer patients, before and after operation. Their data revealed that levels of SCF were increased in 79% of these cases. Results suggest that SCF may be useful in the diagnostic and monitoring of patients with NSCLC (20). Serum stem cell factor and its soluble domain levels were estimated in patients with chronic renal failure (CRF) and anemia and compared with clinical parameters and renal function (13). It was shown that SCF in CRF patients were 5-fold higher than those in healthy controls but for the c-Kit the difference was slight. SCF levels were significantly correlated with blood urea nitrogen. These data showed that the SCF levels increased with the deterioration of renal functions and might be related to erythropoiesis (13).

Prediction of prostate and perhaps bladder cancers metastasizing to the bone and the bone marrow, suggests that the bone and/or bone marrow-derived factors may promote these malignant cells growth or survival at the sites, or are served as chemo-attractants. Savarese *et al.* (21) screened three prostate carcinoma cell lines, DU-145, PC-3, and LNCaP, for the expression of several hematopoiesis - associated colony - stimulating factors (SCFs) and their receptors using RT-PCR (reverse transcriptase-polymerase chain reaction) and immunohistochemical methods, and examined their functional effects. As a result, they showed all of these cell lines express (GM-SCF), (M-SCF), DU-145 and PC-3 stem cell factor. All of these cell lines possess receptors for SCF, GM-SCF, M-SCF, (G-SCF). These data suggest that the mentioned growth factors may be the part of a network of paracrine and autocrine loops modulating prostate carcinoma cell activity. Chemo-tactic actions of bone marrow-derived growth factors on prostate or other carcinoma cells, may thus explain in part why bone is a preferential site of these carcinoma metastases (21).

In all malignancies, early diagnosis plays the most important role in treatment of the patients, and also to prevent the progress of the diseases. Unlike the other urological cancers, bladder cancer lacks most clinically useful biomarkers for predicting disease stage and evaluation of treatment. Studies about systemic circulating levels of neoplastic cell related indicators such as specific proteins interleukins and growth factors, have designated a potential clinical application for determination of these indicators (22). On the other hand, SCF can have evident effects on the various disorders severity and cancers (19–27). In this way, finding the indicatory factors related through the pathological indicator and grade of malignancy is reasonably, the case of interest. The main goal of this approach is determining the range of SCF levels in serum of the bladder cancer patients, and examining its possible relation with cancer stages and pathological indicator.

MATERIALS AND METHODS

Subject preparation

Thirty-five bladder cancer patients were recruited from outpatients at Imam Hospital, Urmia University of Medical Sciences. Only 3 of the patients were female (8.5%). Eleven of these patients had other diseases including rheumatism, hypertension, bladder stone, thyroidectomy, chronic renal failure (CRF), benign prostatic hyperplasia (BPH), and epilepsy. Changes in the SCF level have been defined in only one of them, CRF. The SCF level in the patient with CRF was 2415 pg/ml (mean±SD), that is 2.3-fold higher than normal range. Because of the discrepancy that this high level could induce in the result, it was rejected from the calculation of the patient's SCF level. Bladder cancer patients

were divided into 2 groups according to the presence of samples of prior and post-surgery. First group comprised samples (n=15) from male patients before and after surgery (age 63.5±11.3 years, mean±SD). Post-surgery samples were collected within 2–3 months after operation. Second group contained samples from pre-surgery patients (17 males and 3 females, age 60.3±8.7 years, mean±SD). Control group included thirty-five healthy individuals, (3 females and 32 males, age 61.3±10.1 years). Three patients with CRF were studied as positive controls, since positive relation of CRF with serum SCF level has been studied previously (13).

SCF measurement

Serum SCF level was measured using an ELISA kit (IBL Company, Hamburg, Germany). This kit is based on a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as coloring agent (Chromogen). The potency of coloring was in proportion to the quantities of Human SCF. The precision accuracy and standard used in this assay were conducted as follows; the intra-assay (n=20) and inter-assay (n=20) coefficients of variation (CV) were <4.0% and <10.3%, respectively. Detection limit of SCF kit was 2.0 pg/ml. Each determination was based on a single analysis.

Calculation of SCF concentration

The absorbance of blank samples generated from standard curves and samples were subtracted before plotting standard or data calculation. The standard curve was plotted using eight series concentration of SCF, 0, 50, 100, 200, 400, 800, 1600, and 3200 pg/ml. Best smooth curves were drawn through these points to construct the standard curve. The concentration for unknown samples was then read from the standard curve. Figure 1 shows standard calibration curve for SCF in the range of 50–3000 pg/ml.

Statistical analysis

Analysis were performed with SPSS version 11.0 for Windows. The statistical significance of differences between groups was determined using an unpaired t-test. Distribution of all continuous variables was determined using one-sample Kolmogorov-smirnov test. P<0.05 was considered statistically significant. Clinical and pathological stage was evaluated with and without muscular invasion (MI), clinical and pathological tumor indicator, was evaluated as grades I, II, III and IV.

RESULTS

The concentration of stem cell factor was measured in bladder cancer patients and controls. At first, a comparison was carried out between SCF serum level of bladder cancer patients before surgery and the healthy controls. The results for serum SCF levels were 1049±178 pg/ml (n=35, mean±SD) and 1002±367 pg/ml (n=30, mean±SD) respectively. Secondly SCF serum levels of 15 matched individuals of the previously screened patients were examined before and after surgery. In this case SCF serum levels were 1138±114 pg/ml (n=15, mean±SD) before surgery and 1022±220 pg/ml (n=15, mean±SD) after surgery. Serum SCF levels in patients before surgery were not significantly different from those in normal subjects (p=0.5, Figure 2). Furthermore, no significant difference was found before and after surgery SCF levels in the selected patients (p=0.889, Figure 3). The serum SCF level was also compared between patients and normal individuals, after surgery but no significant difference was found (p=0.92). Secondary, SCF level was also compared in the patients when they are divided according to their cancer stage. SCF level in the patients without muscular invasion (–MI) was 1057±160 (n=21, mean±SD) and in those with muscular invasion (+MI) was 1023±213 (n=12, mean±SD), but the difference in serum SCF levels between –MI and +MI patients were not statistically significant, (p=0.60, Figure 4). Serum SCF level in the grade I patients was

Figure 1: The standard calibration of SCF, the selected range is 50 up to 3000 pg/ml.

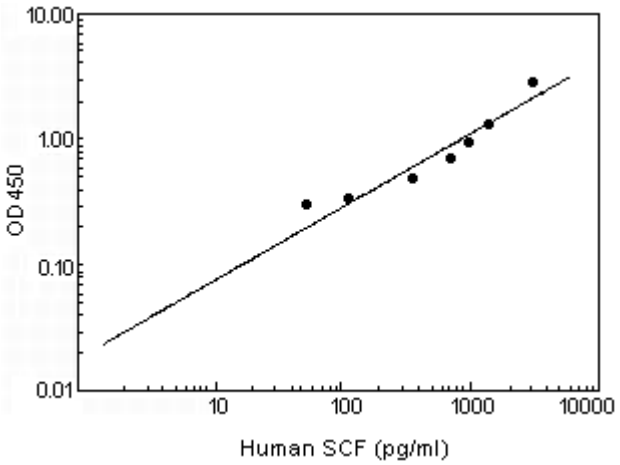


Figure 2: Comparison of serum SCF levles in the patients before surgery (P-BS) and controls.

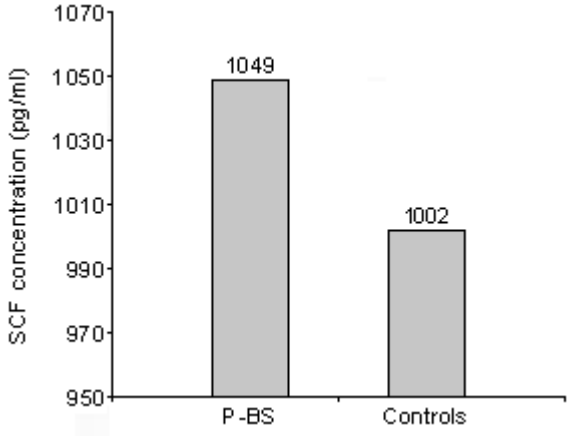
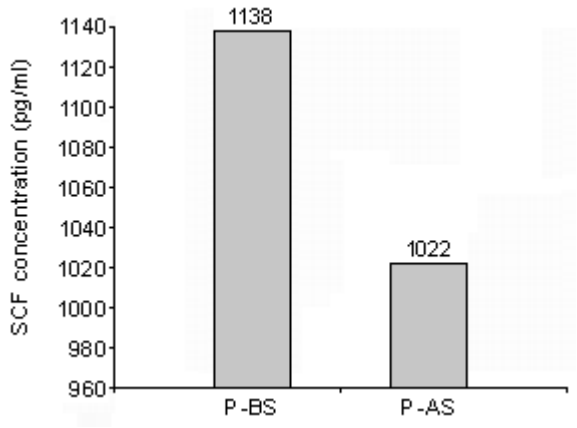


Figure 3: Comparison of serum SCF levles in the patients before surgery (P-BS) and after surgery (P-AS).



1129.2±148.8 pg/ml (n=6, mean±SD), in the grade II patients was 1025.2±164.2 pg/ml (n=17, mean±SD), in the grade III patients was 1023.4±211.9 pg/ml (n=10, mean±SD), and in the grade IV patients was 1150±260.2 pg/ml (n=2, mean±SD), (Table 1). Because of the low number of cases in each grade, we could not compare SCF level regarding to tumor grade. Additionally, we insert smoking, as a parallel influential factor, which could be effective in pathological affairs of this malignancy. In this study, 87% of male patients were smokers that 78% of them smoke more than 2 packs per day. SCF levels in smokers were 1050±179 and in non-smokers were 1048±154 (p=0.90) (Figure 5). These data showed no significant relationship between smoking and SCF concentration. In the last experiment, we examined the CBC count and some of biochemical tests, as probable factors, contributing in the whole procedure. These parameters were measured in the patients before surgery (Table 2). The included parameters could be listed as:

Table 1: Comparison of SCF level in the bladder cancer patients in tumor grades.

Types of grade	n	SCF (pg/ml) (Mean±SD)
Grade I	6	1129.2±148.8
Grade II	17	1025.2±164.2
Grade III	10	1023.4±211.9
Grade IV	2	1150±260.2

hemoglobin (Hb) rates, WBC, RBC and platelet count, neutrophile, lymphocyte, monocyte, eosinophile and basophile count and percentage, BUN and creatinine (Cr) levels (Table 3). None of these parameters were out of their normal range. WBC count was $8.57\pm1.69 \times 10^6$/ml (p=0), RBC count was $4.42\pm0.9 \times 10^9$ /ml (p=0), platelet (PLT) count was $266\pm60 \times 10^6$/ml (p=0), hemoglobin (Hb) level was 13.68±2.50 g/dl (p=0), BUN level was 15.7±5.3 mg/dl (p=0), and creatinine (Cr) level was 1.5±2.1 mg/dl (p=0.006). All these values are within the normal range.

DISCUSSION

In the present study we have measured serum SCF level in bladder cancer patients before and after surgery to elucidate its possible relationship with bladder cancer clinicopathological features. The mean serum SCF levels in normal subjects described by Langley *et al.* (28) was 3300 pg/ml, which was approximately 3.3-fold higher than that in our study. Meanwhile Kitoh *et al.* (13) who also used sandwich ELISA system, described that the normal range was very close to our results (979±181 pg/ml) (29). This discrepancy may be caused by different assay methods which were used. It has been reported that measurement of SCF levels and its function were carried out in bladder carcinoma cells in variant cancer stages (13, 28, 30). Steube *et al.* (30) has reported that the human bladder carcinoma cell line KU-19-19 synthesizes and secretes hematopoietic growth factors, including granulocyte colony-stimulating factor (G-SCF; >5 ng/ml),

Figure 4: Comparison of serum SCF levels in two stages of bladder cancer.

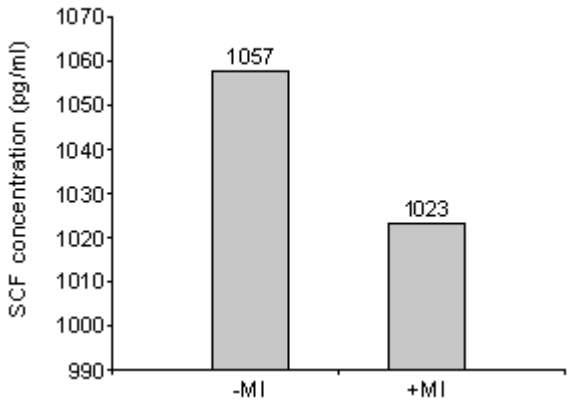
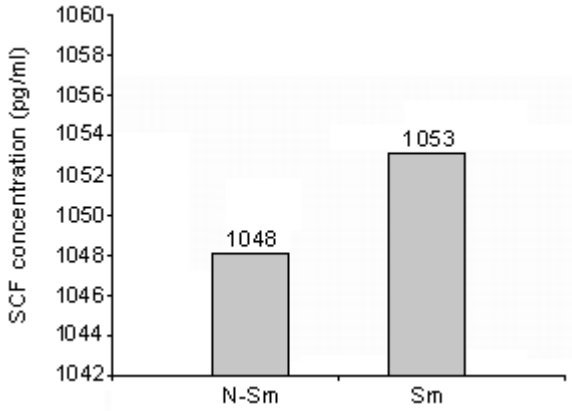


Figure 5: Comparison of serum SCF levels between smokers and non-smoker patients.



granulocyte macrophage colony-stimulating factor (GM-SCF), and stem cell factor (SCF). Results showed that KU-19-19 cell line represents a reliable source for purification of G-SCF and SCF. In regard to this ability of responding to different stimuli, they suggested that there might be regulatory pathways that could be involved in cytokine and growth factor production in this bladder carcinoma cell line (30).

Quentmeier *et al.* (27) showed that the human bladder carcinoma cell line 5637 secrete cytokine in conditioned medium. They used growth factor-dependent cell line bioassays and specific ELISA. The enrolled quantities, which were achieved through specific ELISA assay and by using bioassays, detected high amounts of G-SCF and GM-SCF. On the other hand the data shows smaller quantities for IL-1beta, M-SCF and especially for SCF. In 5637 CM, the concentration of IL-3 was below the detection level of the ELISA (34). There are some other approaches studded in 2000, by Smack *et al.* (23), c-Kit receptor and kit ligand (KL) expression, in prostate cancer were considered and RT-PCR and ISH techniques were used. In this challenge they showed that SCF is normally expressed in prostate tissues, but there is an altered pattern of SCF in the prostate cancer cell line LNCaP and DU-145 and PC-3 (23). The majority of cultured cell lines of epithelial and stromal origins, displayed considerable levels of KL. In addition all cultured prostate cell lines showed significant levels of KL transcripts. All data from this study, reveal that altered patterns of c-Kit and KL expression are associated with BPH (benign prostatic hyperplasia) and adenocarcinoma of prostate. In this way, it seems that SCF factor and its expression of receptors are usually in close correlation with bladder cancer and other related carcinomas. Manegold *et al.* (19) surveyed SCF levels in HIV cases, using ELISA system. They showed the SCF range elevated in patients in stage A as compared with normal controls, but stroke down in stages B and C. Serum levels greater than 1.8 ng/ml were as

Table 2 : Comparison of leukocyte differential parameters in patients with bladder cancer.

Cell types	(%) Mean±SD in the patients	% Normal range	Mean±SD in the patients (cell x 10^6/1)	Normal range (cell x 10^3/1)
Neutrophile	68.7±6.12	55–67	5.9±2	3–5.8
Lymphocyte	22.53±4.9	22–33	1.81±0.53	1.5–3
Monocyte	5.56±2.22	3–7	0.79±0.31	0.28–0.5
Eosinophile	2.18±0.97	1–3	0.22±0.24	0.05–0.25
Basophile	1±0.4	0–0.75	0.05±0.03	0.015–0.05

Table 3: Comparison of clinical parameters, WBC, RBC, Hb, PLT, BUN and creatinine in patients of bladder cancer.

Types of cell	Mean±SD	Normal range
WBC (x10^6/ml)	8.57±1.69	11–4.5
RBC (x10^9/ml)	24.4±0.9	6–4.2
Hb (g/dl)	13.68±2.50	17.5–13.5
PLT (x10^6/ml)	266±60	450–150
BUN (mg/dl)	15.7±5.3	25–8
Cr (mg/dl)	1.5±0.6	1.6–0.6

associated with a longer survival in HIV type 1 seropositive patient (19). Weaver *et al.* (31) has reported that SCF in combination with filgrastim following chemotherapy enhanced the mobilization of progenitor cells in ovarian cancer patients. In conclusion it was revealed that SCF (15 or 20 μg/kg) in combination with filgrastim following chemotherapy is an effective way of increasing progenitor cell yields, following to chemotherapy (31). Esposito *et al.* (25) had an investigation about the possible roles of stem cell factor-c-Kit system possible roles, in pancreatic cancer. In this order, they examined cancerous human pancreatic tissues and 6 cultured pancreatic cancer cell lines for mast cell distribution. In addition, the effects of SCF and of the c-Kit tyrosine-kinase inhibitor STI571, on the growth of the cancer cell lines and of the normal pancreatic ductal cell line TAKA-1, were assessed (25). Their results as showed SCF immunoreactivity were absent in acinar, ductal, and islet cells of the normal pancreas and faint in pancreatic cancer tissues and cell lines. In contrast, in some normal and hyperplastic ducts tissue of the normal pancreas and in the cancer cells, 73% of the tumor samples, and in all the cell lines had been tested, c-Kit was clearly present. SCF showed a dose-dependent growth inhibitory effect on TAKA-1 cells ($p<0.001$), whereas pancreatic cancer cells were resistant to the SCF-induced growth inhibition. As a conclusion, the SCF-c-Kit system, possibly with the contribution of mast cells, may have a growth-regulating role in the normal pancreas, which is altered during malignant transformation. It seems that up to now, determining of soluble stem cell factor serum levels in bladder cancer, is not considered in any approaches and there are few studies focused on related cases.

Our results showed that after surgery serum SCF levels were slightly lower than before surgery. SCF levels were faintly more in bladder cancer patients than in healthy controls. But it could not be demonstrated, to have any statistical correlation in each case. Our results showed that SCF levels had not any correlation with smoking, grade and stage of disease, and there was no association between SCF levels with Hb, BUN, creatinine levels, and WBC, RBC and PLT count as well, and also were for neutrophile, lymphocyte, monocyte, eosinophile and basophile count and percentages. Consequently, it is considered that, maybe serum SCF level could not be a useful marker for clinical feature determination of bladder cancer. Mast cells and fibroblasts accumulate within solid tumors (including bladder tumors) and can release many angiogenic factors (including stem cell factor), and act in vascularization (9, 32). Many established data showed that stem cell factor acts in fluency for cell migration and proliferation and degeneration of fibroblasts (9, 33), so we suggest further investigation, which would examine these subjects in bladder cancer, must be carried out. On the other hand, it has been shown that urinary concentration of IL-2, IL-1 and IL-6, TNF in bladder transitional cell carcinoma patients, are similar to dose of healthy controls (34). Thus, it is not fairly rational, to think maybe, the lack of significant increase in soluble growth factors, could be due to excretion in urine. Further researches are necessary to elucidate that absence for significantly increase in serum SCF levels, could be concluded from its low production in tumor or whether if some transient cases of inhibitory. Assay of SCF mRNA amount in bladder tumor cells, and analyze for membrane SCF amount in bladder tumor cells, are some of those subjects, than could be suggested for further investigations. Confirmation of these results in wide ranged prospective studies is also seems to be needed.

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