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The role of TGF Beta 1 in renal cell

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Abstract
Background: *Transforming growth factor-β (TGF-β) is a multifunctional cytokine that has been linked to vascular remodeling processes, myocardial hypertrophy, and renal fibrosis. The aim of the present study was to evaluate the clinical value of tgfβ1as a tumor marker for the diagnosis and postoperative follow-up of patients suffering from RCC.*
Methods: *A total of 36 patients were included in the study. These patients were first evaluated for RCC and then later confirmed by HPE. tgfβ1 in plasma samples was estimated by using a commercially available ELISA kit From DRG INTERNATIONAL, Inc GmbH Germany.*
Results: *After HPE clear cell type was the most common type (25/32 -78.1%), followed by papillary type (4/32 -15.6%), chromophobic type (2/32 -6.2%) and angiomyolipoma (1/32 -3.1%). Out of 36 patients 29 (80.5%) patients had no lymph nodes and 7 (19.4%) patients were positive for lymph nodes. 36 Patients of RCC showed elevated level of tgfβ1as compared to the control group. Comparing these tgfβ1 levels with the localized RCC group having mean levels 25527.42 ng/l, a statistically significant difference (P<0.05) was found.*
Conclusions: *The present study indicates that tgfβ1 levels in RCC patients irrespective of pathological stage of tumor & or metastasis remain quite high even after nephrectomy & even upto six months of follow up. Thus tgfβ1 levels alone cannot be used as single prognostic marker to monitor the progression of disease or treatment in RCC.*
Keywords: *Transforming growth factor-β1 - diagnosis - monitoring – prognosis, renal cell carcinoma (RCC).*

Introduction

Transforming growth factor-β (tgfβ) belongs to a family of dimeric 25-kDa polypeptides that are ubiquitously distributed in tissues and synthesized by many different cells.[1,2] In mammalians, three isoforms are found: tgf-β1, tgf-β2, and tgf-β3. All three are cytokines with multiple functions, e.g., regulation of cell proliferation and differentiation,

promotion of wound healing, and suppression of the immune system. While many cells are able to synthesize tgf-β, the most prominent sources within the body are bone matrix and the α-granules of platelets.[2] Cells secrete tgf-β in a biologically inactive, latent form bound to an amino-terminal propeptide called latency-associated peptide (LAP). Transient acidification is commonly used to activate this so-called small, latent tgf-β (Ltgf-β), i.e., to release tgf-β from its noncovalent association with LAP. Numerous other proteins, including proteoglycans, type IV collagen, fibronectin, and other components of the extracellular matrix have been reported to noncovalently bind tgf-β.[3,4] In blood, tgf-β supposedly occurs associated mainly with α_2-macroglobulin (α2M).[5,6]The determination of tgf-β in blood has been advocated for diagnosis of various diseases, e.g., cancer,[7,8] immunological disorders[9] and hematological[10] or fibrotic diseases.[11]

To date only a few tumor markers for RCC have been evaluated.[12-15] However, due to comparatively low sensitivity levels, these markers are not applicable for routine diagnosis. Usually only detailed histopathological examination and the analysis of the primary tumor is available for limited prognostic information, with the pT-stage being the most useful parameter. However, plasma/serum tumor markers with high sensitivity and specificity would be useful in order to provide adequate monitoring of the patients following radical nephrectomy or immunochemotherapy and for the early detection of metastatic disease.

Material & Methods

A total of 36 patients were included in the study conducted at Sheri Kashmir Institute of Medical science Soura Srinagar for the period of two years. These patients were first evaluated for RCC and then later confirmed by HPE. The patients were also evaluated for their susceptibility for anaesthesia and for undergoing surgery. The 1st sample was withdrawn before surgery after which patients were then subjected to nephrectomy and the loin incision was preferred. The specimen retrieved was sent for HPE. On the 3-5 post-operative day 2nd sample was taken. The patients were on follow up for 2-6 months during which the third sample was taken. These patients were also screened by radiology and other biochemical parameters for any evidence of recurrence or residual disease. Also samples were collected from n=80 age and sex matched controls who were relatives of the patient.

tgfβ1 in plasma samples was estimated by using a commercially available ELISA kit From DRG INTERNATIONAL, Inc GmbH Germany. It is a solid phase enzyme linked immunosorbent assay (ELISA) based on the sandwich principle. The assay was carried out following the manufacturer's protocol. Blood samples from patients were taken by venipuncture following universal precautions and all aseptic conditions. Briefly 5ml of blood samples were drawn in lavender top tubes and blood was mixed with EDTA by inversion. The tubes were then centrifuged at 2000rpm using a Remi centrifuge for 5 minutes. The clear plasma so obtained was aspirated by using disposable plastic droppers and dispensed in 2 ml screw capped vials. The plasma vials were stored at -20^0Ctill assayed for tgfβ1 levels.

The study was approved by Institute ethical committee. The patients were enrolled for the study after taking proper consent from each of them individually

Test Procedure

The steps followed for estimation of tgfβ1 levels were as follows.

The desired number of coated microtitre wells were secured in the frame holder.100 ul of each pre-treated, Standards, Controls, and Samples were dispensed with fresh disposable tips into appropriate wells. The plate was covered and incubated overnight for 16 hrs. at 4°C. The contents were briskly shaken out the of the wells and the whole plate was washed three times with diluted wash solution (300 micro litre per well)

using an automated plate washer. After removing all moisture from the wells by striking on the absorbent paper, 100 microlitre antiserum was dispensed into all wells and incubated for 120 mins at room temperature. Washing step was repeated as described above. 100 microlitre enzyme conjugate (anti mouse biotin)was then dispensed into each well and the plate was Incubated for 45mins at room temp. Washing step was repeated as described above and 100 micro litre enzyme complex was then added to each well. The plate was incubated again for 45mins at room temp. After repeating the washing procedure 100 microlitre of substrate solution was added to each well and the plate was Incubated for 15 mins at room temp. under dark cover. Enzyme substrate reaction was stopped by adding 50 microlitre of stop solution (1 N HCL) to each well. The absorbance of each microtitre well was read at 450nm using a programmable ELISA reader from Bio Rad laboratories. The requisite information as provided by the kit insert regarding standards and the method of calculation(Semi log curve) was used in the reader for determining the values of patient samples.The values of tgfβ1levels so obtained in samples were recorded and correlated with the patient details and pathological reports.

Results

A total of 36 patients were subjected to evaluation for tgfβ1. Demographic characteristics are shown in table 1.

characteristics	N=36 (%)
Male/ Female	24(66.6%) 12(33.3%)
Age (≥50) (<50)	33(91.6%) 3(8.3%)
Smoking (smokers) (Non smokers)	24(66.6%) 12(33.3%)
Side (Right/ Left)	21 (58.3%)15 (41.6%)

Table 2: Presenting symptom

Variable	Parameter	N=)36 %)
Symptom	Flank pain	20 (55.5%)
	Haematuria	19 (52.7%)
	Abdominal lump	6 (16.6%)
	Triad	2 (5.5%)
	Other	11(30.5%)

On the basis of presenting symptom 20 (55.5%) patients presented with flank pain, 19 (52.7%) patients with hematuria,6 (16.6%)with abdominal lump and 11 (30.5%) patients had other symptoms .The classical triad of hematuria, flank pain and abdominal lump was seen in only 2 patients (5.5%).

Table 3: Histopathological Stage and Pathological type:

Variable	Parameter	N=36.n%
Stage	Stage I	2(5.6%)
	Stage II	4(11.1%)
	stage III	10(27.8%)
	stage IV	20(55.6%)
Pathological type	Clear cell	25(78.1%)
	Papillary	4(15.6%)
	Chromophobic	2(6.25%)
	Angiomyolipoma	1(3.1%)

Based on stage of the tumour the breakup was stage I (2 patients-5.6%), stage II (4 patients-11.1%), stage III (10 patients-27.8%) and stage IV(20 patients-55.6%).

In HPE clear cell type was the most common type (25/32 -78.1%), followed by papillary type (4/32 -15.6%), chromophobic type (2/32 -6.25%) and angiomyolipoma (1/32 -3.1%).fig.1

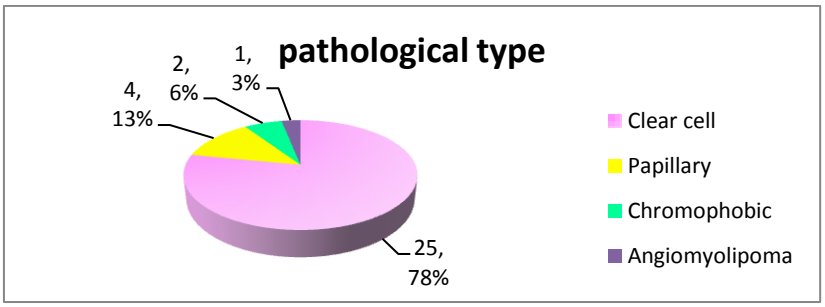

Fig.1

36 Patients of RCC showed elevated level of tgfβ1. Mean preoperative plasma concentrations of 27930 ng/l (18,380–46,530 ng/l) fortgfβ1. In comparison, the age and sex matched control group (n=80) had mean plasma concentration of 2584 ng/l (1,853-3,315 ng/l). fig.2

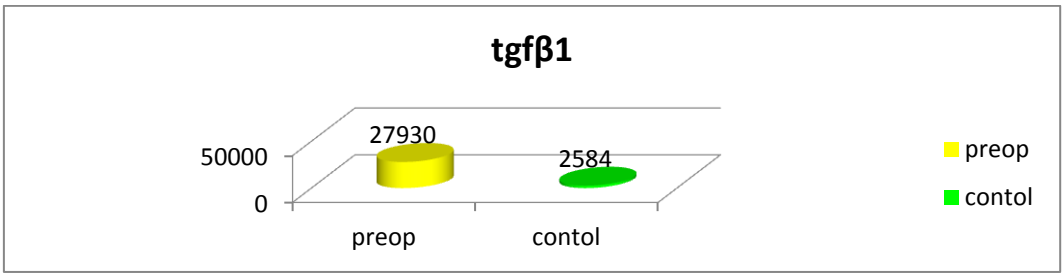

Fig 2

tgfβ1 levels after surgical therapy the post nephrectomy group had mean concentrations of 26892 ng/l (12,425–46,380 ng/l) for tgfβ1.The differences between these two groups were not statistically significant. Mean plasma levels during follow-up without evidence of disease (2–6 months) were 8878.52ng/l (4,526-15,615 ng/l); these levels showed differences but did not normalize.

Mean preoperative and post-operative levels in stage III were compared and the results were non significant.fig 3.

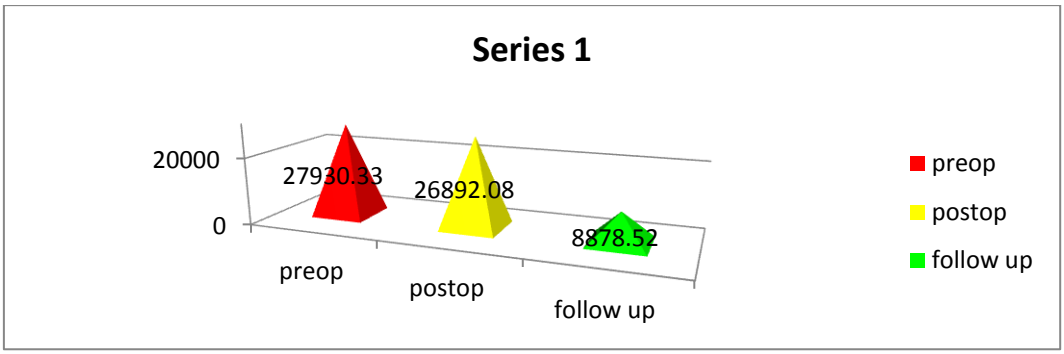

Fig 3

No significant differences between the pathological subgroups was found after stratifying the patients using the UICC TNM-system.fig 4

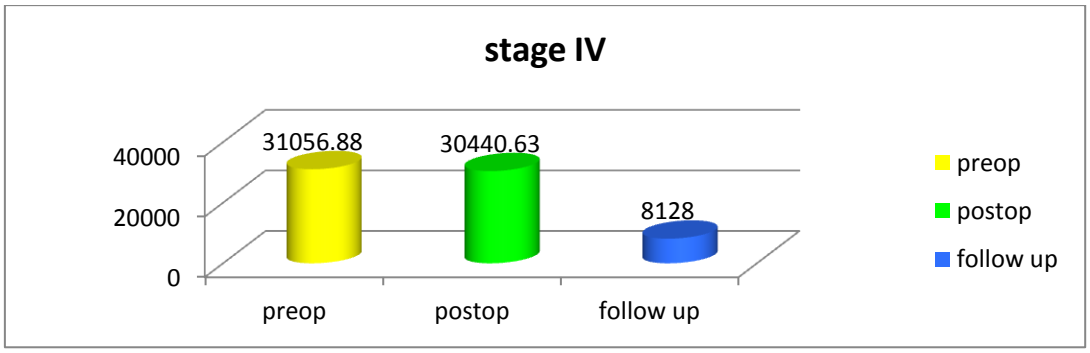

Fig.4

Patients with metastatic disease showed mean plasma concentrations of 42828.40 ng/l fortgfβ1. Comparing these tgfβ1 levels with the localized RCC group having mean levels 25527.42 ng/l, a statistically significant difference ($P<0.05$) was found. No difference was found between the different locations of the metastases.fig 5

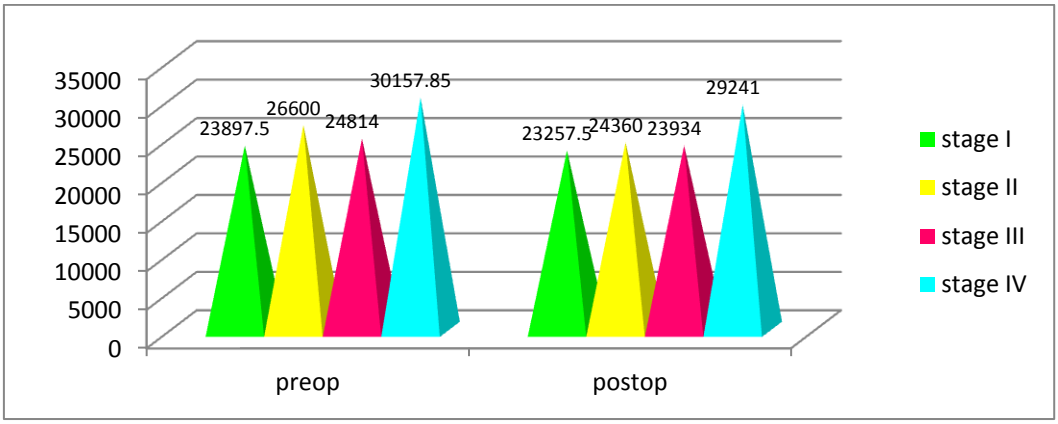

Fig 5

Discussion

Cytokines are multifunctional peptides with many biological activities, being synergistic or antagonistic with one or more functions. Several potent immunomodulatory cytokines, such astgfβ1 or IL-6, are produced by proximal tubular cells or cell cultures derived from RCC of proximal tubuli origin (conventional RCC) .These cytokines may exert tumor-promoting and immunosuppressive effects .tgfβ1 is one of the mammalian gfβ isoforms which are homologous peptide growth factors that act by binding to a single common receptor complex. Tgfβ family proteins are implicated in many biological processes including embryogenesis, tissue repair, and the regulation of haematopoiesis and the immune response. An interesting feature is that tgfβ1 family proteins are potent growth suppressors of different normal cell types, while, on the other hand, many tumors are resistant to tgfβ1. The clinical utility of tgfβ1 has been implicated in various cancers like breast, colon, prostate and hepatocellular carcinoma. [16-19]

The aim of the present study was to evaluate the clinical utility of tgfβ1 plasma-levels in patients suffering from RCC in order to obtain a prognostic reference and to facilitate postoperative monitoring.

The role of tgfβ1 was also evaluated by Hegele et al (2002),[12] Junker et al[20] (1996,1997) and Wunderlich, etal (1997,1998).[21]

To determine the amount of tgfβ1 levels an optimized activation procedure was applied.[22]

We analysed plasma samples from 36 RCC patients having mean age of 58yrs having a male: female ratio of 24(66.66%) /12(33.33%)for tgfβ1 levels both pre & post operatively and on follow up of 2-6 months of different pathological stages based on TNM staging system. A control group was taken of 80 age and sex matched healthy controls.

Our observation was that the tgfβ1levels remained quite high in patients of RCC as compared to healthy controls. This observation has been confirmed by Hegele et al (2002), Wunderlich, et al (1998) and Junker,et al in their study.[12,20]

Wunderlich, etal (1998)[21] in his studyalso determined tgfβ1 plasma concentrations and described elevated levels in 20 patients suffering from RCC by using an ELISA .In their study the researchers compared tgfβ1 levels from patients with RCC before they underwent radical nephrectomy, with levels from patients with extracorporeal lithotripsy, pyelonephritis, and healthy controls. They found that the tgfβ1 plasma levels in both the groups were found to be higher than in healthy controls. But the tgfβ1 levels were significantly higher in RCC patients than in cases of inflammation and concluded that tgfβ1 is a possible tumor-prognostic marker in RCC. In 1996 Junker et al measured the levels of tgfβ1in plasma samples by enzyme-linked immunoabsorbent assay (ELISA). Samples were collected from patients suffering from renal cell carcinoma (RCC) before they underwent tumour resection. In all cases tested, the levels of latent

tgfβ1 were much higher than in healthy controls. He concluded that that elevated latent tgfβ1 is common in RCC, is at least partially produced by the tumour and might be a cause for local immunosuppressive effects within the tumour. Similar results were observed in the present study also. In 2002 Hegele et al[12] in his study compared the tgfβ1levels between patients of RCC and the patients of other non malignant urological disease and found no significant difference between the two groups. Further in their study the plasma levels after operative therapy (days 1, 5 and 10) and during follow-up without evidence of disease (2–6 months) showed no significant differences.

We in our study compared the preoperative tgfβ1 levels with the post nephrectomy group. The results obtained were not statically significant which is in line with Hegele et al (2002).[12]

During the follow period our study did show regression in tgfβ1 levels, but the levels remained persistently high and never normalised without the evidence of any localised disease based on postoperative clinical features radiological evidence and biochemical parameters.

In our study based on stage of the tumor the breakup was stage I (2 patients-5.6%) , stage II (4 patients-11.1%) ,stage III (10 patients-27.8%) and stage IV(20 patients-55.6%). Intra stage comparison with respect to tgfβ1 levels; No significant differences between the pathological subgroups was found after stratifying the patients using the UICC TNM-system which is supported by the authors.

In comparison to the localized RCC group patients with metastatic RCC, a statistically significant difference was found between tgfβ1 levels. In our study we also observed very high levels of tgfβ1 levels in patients having metastasis than in patients who had localized disease. Hegele et al (2002)[12] also reported similar findings in their study. This aspect is of great interest for the follow-up after surgical therapy and the response to subsequent immunochemotherapies in metastatic disease. Although, there is no doubt that tgfβ1 plays an important role in the neovascularization of RCC; results by Hegele et al (2002)[12] demonstrated that the evaluation of tgfβ1 plasma levels in patients with localized RCC had no diagnostic value. Our results also support this finding. It seems that tumour cells are not the predominant source of tgfβ1 production within the organism and that tumor cells also do not stimulate tgfβ1 output by other tissues.

We are of the opinion that measuring tgfβ1 levels in plasma is not a suitable tumor marker alone for the diagnosis of localized RCC. Whether tgfβ1 might be useful for the early detection of RCC recurrence, to control the success of immunochemotherapy in metastatic disease or as a prognostic parameter in the face of higher plasma levels in metastatic disease has to be proved by further research.

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