Insulin-like growth factor-1 and childhood cancer risk

MOHAMED BADR[1], TAMER HASSAN[1], SHEREEN EL TARHONY[2] and WAEL METWALLY[1]

Departments of [1]Pediatrics, and [2]Medical Biochemistry, Zagazig University, Zagazig, Egypt

Abstract. Overexpression of growth factors and/or their receptors is a common event in malignancy and provides the underlying mechanisms for one of the hallmarks of cancer, uncontrolled proliferation. Mounting evidence suggests that IGF-1 is involved in the pathogenesis and progression of different types of human cancer such as colon, breast, prostate and lung. However, only a few studies have investigated the association between IGF-1 levels and childhood cancer risk. We aimed to compare the IGF-1 serum level in children with *de novo* malignancies to healthy children, and to assess its relationship with cancer type, stage, metastasis and different disease characteristics. The study was carried out on 100 children; 50 children with *de novo* malignancies and 50 healthy children of matched age and gender as a control group. The patients were subjected to a routine work-up for their cancers according to our local standards. Estimation of the serum level of IGF-1 was carried out in the two groups using ELISA. Our results showed that children with cancer had significantly higher levels of IGF-1 than healthy controls of the same age and gender. No association was found between IGF-1 and tumor type, stage, metastasis and other disease characteristics. In conclusion, the IGF-1 serum level is an important indicator of risk for the most prevalent forms of childhood cancer. It may be used to identify children at the highest risk for these cancers and aid in determing who may benefit most from preventive strategies. Given the small number of children in our study, studies with larger populations are required to confirm these results.

Introduction

Insulin-like growth factors (IGFs) are polypeptides with a high sequence, similar to insulin. IGFs are part of a complex system that cells use to communicate with their physiologic environment. This complex system (often referred to as the IGF 'axis') consists of two cell-surface receptors (IGF1R and IGF2R), two ligands (IGF-1 and IGF-2), a family of six high-affinity IGF-binding proteins (IGFBP-1 to -6), as well as associated IGFBP-degrading enzymes, referred to collectively as proteases.

It has become increasingly clear that the growth hormone (GH)/IGF-1 axis plays a fundamental and obligatory role in regulating normal somatic growth throughout fetal and childhood development. Over the past two decades considerable evidence has accumulated showing that these growth factors play an important role in maintaining and supporting the progression of neoplastic growth. A number of epidemiological reports showed that it may also be an important determinant of cancer incidence (1).

The risk of cancer is higher among people with elevated concentrations of IGF-1, and is lower among those with high concentrations of IGFBP-3 (the main binding protein). The associations are similar when people whose blood samples were taken shortly before diagnosis are excluded from analyses, suggesting that the observed correlations are not due to the release of the growth factor by preclinical cancers (2).

In adults, numerous studies demonstrated that higher IGF-1 levels are associated with a significantly increased risk of developing a number of the most common types of cancer, such as colon, breast, prostate and possibly lung (3). However, only a few studies have investigated the association between IGF-1 levels and childhood cancer risk. Higher levels of IGF-1 were shown to increase the risk of leukemia in children (4). Additionally, a role has been demonstrated for IGF-1 and other components of the IGF system in the pathogenesis and progression of other childhood malignancies (5).

IGF-1 exerts powerful effects on each of the key stages of cancer development and behavior: cellular proliferation and apoptosis, angiogenesis and metastasis, and more recently, development of resistance to chemotherapeutic agents (6,7).

Given the mounting evidence of the risk of cancer, caution should be exercised in the exogenous use of either IGF-1 or substances that increase its concentration (8).

This study aimed to compare the IGF-1 serum level in children with *de novo* malignancies to healthy children, and to assess its relationship with cancer type, stage, metastasis and different disease characteristics.

Subjects and methods

This study was conducted in the Pediatric Hematology and Oncology Unit of Zagazig University Hospital and the Department of Medical Biochemistry, Faculty of Medicine,

Correspondence to: Dr Tamer Hasan Hassan, Department of Pediatrics, Faculty of Medicine, Zagazig University, 44111 Zagazig, Egypt
E-mail: dr.tamerhassan@yahoo.com or thhassan@zu.edu.eg

Table I. Age and gender of the patients and controls.

	Patients n=50	Controls n=50	Value of the test of significance	P-value
Age (years)				
Mean ± SD	2.5±4.9	2.9±4.89	t=0.25	0.82
Range	1-11	1-11		
Gender				
M/F (no.)	27/23	25/25	χ^2=0.16	0.68
M/F (%)	54.0/46.0	50.0/50.0		

Table II. Tumor type in the patient groups.

Tumor type	No.	%
Hematological malignancies	40	80.0
ALL	23	46.0
AML	11	22.0
Biphenotypic AL	2	4.0
Hodgkin's lymphoma	2	4.0
Non-Hodgkin's lymphoma	2	4.0
Solid tumors	10	20.0
Neuroblastoma	3	6.0
Ganglioneuroblastoma	1	2.0
Rhabdomyosarcoma	3	6.0
PNET	1	2.0
Wilms' tumor	1	2.0
Duodenal adenocarcinoma	1	2.0

PNET, Primitive neuroectodermal tumor; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AL, acute leukemia.

Zagazig University, Egypt, during the period from January 2009 to December 2009.

Subjects. The study included two groups. Group I (patient group) consisted of 50 children with *de novo* malignancies, whether hematological malignancies or solid tumors. Group II (control group) consisted of 50 age- and gender-matched healthy children as a control group.

Methods. Patients were subjected to a routine work-up for their cancers according to our local standards. Estimation of the serum level of IGF-1 in the two groups was carried out using the DRG IGF-1 600 ELISA kit on the Sunrise Remote/ Touch ELISA analyzer.

Specimens. A total of 3 ml of blood was collected by venipuncture and allowed to clot. The serum was then separated by centrifugation at room temperature. Serum samples were frozen at -20°C until the time of assay.

Principle of the test. The DRG IGF-1 600 ELISA kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. Patient samples,
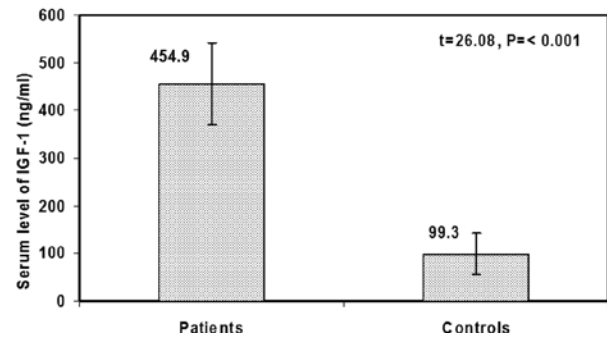
Serum levels of IGF-1 in Patients and Controls.

Figure 1. Children with cancer had significantly higher levels of IGF-1 than the healthy controls.

standards and controls were acidified and neutralized prior to the assay procedure. Microtiter wells were coated with a monoclonal antibody directed towards an antigenic site on the IGF-1 molecule. The pre-treated sample was incubated at room temperature with conjugate (biotinylated IGF-1). The wells were washed and incubated with an enzyme complex (strepta-vidin-HRP-complex). After addition of the substrate solution, the intensity of the developed color was reverse proportional to the concentration of IGF-1 in the patient sample.

Statement of ethics. The present study was conducted in accordance with the ethical standards of the Helsinki Declaration of 1964, as revised in 2000, and was approved by our local ethics committee. Informed consent was obtained from the study participants or their guardians.

Statistical analysis. Data were assessed, entered and analyzed using SPSS version 15. Data were expressed as the mean ± standard deviation for quantitative variables, number and percentage for qualitative variables. ANOVA (F test), the Student's t-test, Chi-square test, Kruskall Wallis (K) test and the correlation coefficient (r) were used when appropriate. $P<0.05$ was considered to indicate a significant difference.

Results

The study was carried out on 100 children: 50 children with *de novo* malignancies and 50 healthy children of matched age and gender as a control group. The age and gender of the two groups are shown in Table I.

The patient group included 40 children with hematological malignancies and 10 children with solid tumors. Acute leukemia was the most common childhood cancer in our patients accounting for 72.0% of all of the cancer types. Acute lymphoblastic leukemia (ALL) accounted for approximately two thirds of the children with acute leukemia. Lymphoma was the second most common cancer type accounting for 8% of the cancer types. Solid tumors collectively represented 20% of all of the cancers. Neuroblastoma and rhabdomyosarcoma were the most common solid tumors in our patient group. Table II summarizes the different tumor types in our patient group and their relative percentages.

Table III. Comparison between different age groups of patients and controls with regard to serum levels of IGF-1.

| Age group (years) | Serum levels of IGF-1 (ng/ml) | | t-value | P-value |
	Patients (mean ± SD) (Range)	Controls (mean ± SD) (Range)		
<2	458.75±63.3 (401-539)	92.8±47.7 (45-173)	10.90	<0.001
2-5	448.2±84.2 (285-605)	97.0±41.5 (40-200)	18.90	<0.001
6-9	457.6±85.7 (299-632)	112.3±52.4 (52-192)	12.38	<0.001
9-11	501±150.9 (331-619)	119.2±32.9 (85-156)	5.73	<0.010

Table IV. Comparison between different gender groups of patients and controls with regard to serum levels of IGF-1.

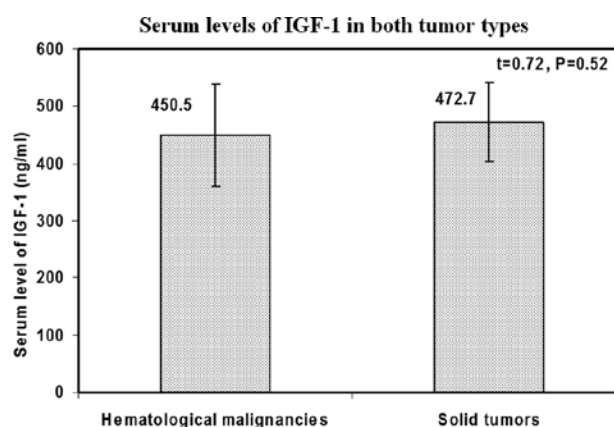| Gender | Serum levels of IGF-1 (ng/ml) | | t-value | P-value |
	Patients (mean ± SD) (Range)	Controls (mean ± SD) (Range)		
Male	457.4±91.0 (285-632)	113.0±48.8 (45-200)	16.80	<0.001
Female	451.4±80.8 (299-619)	92.2±37.5 (40-189)	20.02	<0.001

Figure 2. No statistically significant difference was found between patients with hematological malignancies and those with solid tumors with regard to serum levels of IGF-1.
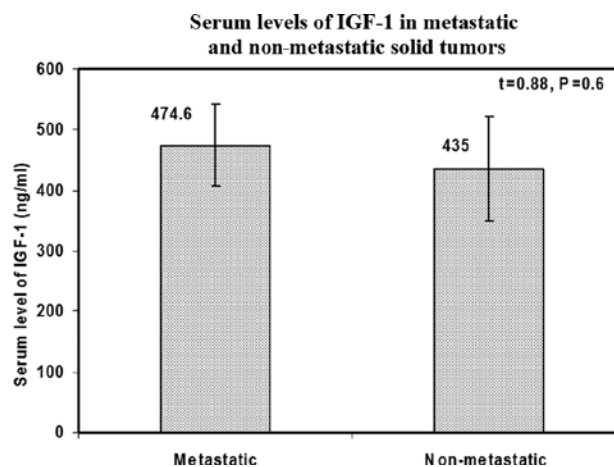

Figure 3. Although the patients with metastatic solid tumors had higher serum levels of IGF-1 than those without metastasis, the difference was not statistically significant.

IGF-1 serum levels in the patients and controls. Our results showed that the children with cancer had significantly higher levels of IGF-1 than the healthy controls (mean ± SD was 454.9±85.7 ng/ml for patients and 99.3±44.1 ng/ml for controls (p<0.001) (Fig. 1). Gender and age are significant factors affecting the serum level of IGF-1. Subsequently, we divided our patients into different age and gender groups and found that the cancer patients still had significantly higher serum levels of IGF-1 than the healthy controls of the same age and gender group (Tables III and IV).

IGF-1 serum levels, tumor type, and initial clinical and laboratory data. No statistically significant difference was noted between patients with hematological malignancies and those with solid tumors with regard to the serum levels of IGF-1 (P=0.52) (Fig. 2). Additionally, no relationship was found between IGF-1 levels and any of the initial clinical and laboratory data including the overall risk of the patients.

IGF-1 serum levels and different ALL immunophenotypes. Although T-ALL patients had higher serum levels of IGF-1 than those with other immunophenotypes, the difference was not statistically significant (P=0.61).

IGF-1 serum levels and different French-American-British (FAB) subtypes in ALL and acute myeloid leukemia (AML). No significant relationship was noted between the serum levels of IGF-1 and FAB subtypes in the patients with acute leukemia (P=0.18 and 0.45, respectively).

IGF-1 serum levels and metastasis in solid tumors. Although patients with metastatic solid tumors had higher serum levels of IGF-1 as compared to those without metastasis, the difference was not statistically significant (P=0.6) (Fig. 3).

IGF-1 serum levels and different stages of solid tumors. No significant relationship was found between the serum levels of IGF-1 and different stages of solid tumors (P=0.23).

Discussion

Since its identification, there have been unresolved concerns about the potential cancer-enhancing properties of IGF-1. Circumstantial evidence in support stems from various sources: *in vitro* studies, animal studies, epidemiological

observations within the general population and patients with growth hormone (GH) excess and deficiency, as well as from the therapeutic manipulation of GH and IGF-1 actions (9).

Considerable epidemiological data have suggested a possible link between circulating GH and/or IGF-1 levels and the development of a variety of different cancer types. Numerous studies suggested that subjects with serum IGF-1 levels that are in the higher percentiles of the normal range have a significantly increased risk of developing a number of the most common types of cancer, such as colon, breast, prostate and possibly lung (3). However, only a few studies have investigated the correlation between IGF-1 levels and childhood cancer risk.

Our results showed that children with cancer had significantly higher levels of IGF-1 than healthy controls. Based on the fact that serum levels of IGF-1 are affected by age and gender, this relationship was examined in different age and gender groups. We found that cancer patients showed significantly higher serum levels of IGF-1 than healthy controls of the same age and gender group.

The relationship between higher levels of IGF-1 and cancer can be explained by the fact that IGF-1 is a potent proliferative agent affecting almost every cell type and also a powerful antiapoptotic agent affecting apoptotic responses to a variety of agents of numerous cell types. These two effects result in a state of hyperproliferation. Such an imbalance between cell proliferation and death would favor, even slightly, the survival of stem cells that had undergone early genetic 'hits'. Thus, the pool of damaged cells available for second and subsequent hits are likely to increase (10).

Whether IGF-1 is a causal factor or simply a surrogate measure of the malignant process remains unknown. A study by Ma *et al* (2) showed an association between colorectal cancer risk in men and elevated plasma levels of IGF-1 by using plasma samples drawn over a long period of time prior to the clinical appearance of the tumors. Thus, the possibility that plasma levels were affected by the disease process was minimized, thereby confirming the causal relationship between IGF-1 and cancer.

Numerous studies have investigated the role of IGF-1 in hematological malignancies and solid tumors (5). However, no study thus far has compared the levels of IGF-1 in the two tumor types.

In our study, no differences were noted between hematological malignancies and solid tumors and between ALL and AML patients with regard to the serum level of IGF-1, confirming that IGF-1 levels are higher in cancer patients regardless of their cancer type. Additionally, no association was found between IGF-1 levels and different FAB subtypes in ALL and AML patients. In ALL patients, no relationship was found between IGF-1 levels and different immunophenotypic subtypes.

High levels of IGF-1 have been shown to increase proliferative stress on progenitor cells in bone marrow (4,11), increasing the number of cell divisions and, in turn, the risk of leukemia. Further evidence supports a role for IGF-1 in the pathogenesis of leukemia: IGF-1 receptors were found to be expressed on leukemic lymphoblasts; IGF-1 stimulated the growth of leukemic cells *in vitro* (12); IGF-1 has been shown to protect hematopoietic cells from apoptosis (13); and the

administration of GH, the effect of which is mediated through the IGF-1 system (14), has been reported to increase the risk of childhood acute leukemia (15).

Accelerated fetal growth may be a risk factor for childhood ALL, a tenet supported by evidence that is related to increased levels of IGF-1. In a recent study, McLaughlin *et al* (16) reported an increased risk of ALL among children with birth weights greater than 3,500 g.

Rangel *et al* (17) also showed that the estimated risk for certain types of cancer has been found to be statistically and significantly higher with a birth weight of more than 4,000 g (The estimated risk was 1.86 for leukemia, 1.99 for non-Hodgkin's lymphoma and 4.76 for Wilms' tumor).

Contrary to our results, Petridou *et al* (14) reported that there was no significant association between IGF-1 and the likelihood of childhood leukemia. However, these authors found that an increment of 1 μg/ml of IGFBP-3 was associated with a substantial and statistically significant reduction in childhood leukemia by 28%. Since IGFBP-3 is essentially a binding protein, these findings suggest that bioavailable IGF-1 plays an important role in the etiology of childhood leukemia.

The role of IGF-1 and other components of the IGF system in the pathogenesis and progression of other childhood malignancies have been investigated (5). In lymphoma, IGF-1 has been shown to stimulate the proliferation of T-cell lymphoma lines that have phenotypic characteristics of thymic pre-T cells and to inhibit cell differentiation, which may crucial in early lymphoma tumorigenesis (18). Moreover, IGF-1 causes a significant increase in the proliferation of Burkitt's lymphoma cells, which may be blocked by antiserum against IGF-1 (19).

IGF-2 plays a role as both an autocrine and a paracrine growth factor in neuroblastoma (20,21). High levels of IGF-1 have been shown to play an important role in the pathogenesis of osteosarcoma (22). Northern blot analysis of tumor biopsy specimens from patients with alveolar and embryonal rhabdomyosarcoma showed high levels of IGF-2 mRNA expression, suggesting the possibility that the upregulation of IGF-2 plays a role in the unregulated growth of these tumors (23). The role of IGF-1R signaling in Ewing's sarcoma family of tumors (ESFT), including primitive neuroectodermal tumors, has been evaluated extensively. EFST cell lines were shown to express IGF-1R and secrete IGF-1. IGF-1R-blocking antibodies were shown to be successful in the interruption of this autocrine loop (24,25). Inactivation of WT1, a tumor-suppressor gene, has been shown to up-regulate a number of components of the IGF system that contribute to inappropriate proliferation and development of Wilms' tumor (26-28).

A role for the IGF system in cancer metastasis has recently been documented in various types of human cancer. Barozzi *et al* (29) found that the overexpression of IGF-2 was predictive of liver metastasis in patients with colorectal cancer. Hakam *et al* (30) showed a stepwise increase in the expression of IGF-1R during the progression from colonic adenomas towards primary colorectal adenocarcinomas and metastases. In our study, although the serum levels of IGF-1 were higher in metastatic solid tumors than non-metastatic ones, the difference was statistically non-significant.

In conclusion, the IGF-1 serum level is an important indicator of risk for the most prevalent forms of childhood cancer and may be used to both identify children at the highest risk

for these cancers and, aid in determining who may benefit most from preventive strategies. Given the small number of children in our study, studies with larger populations are required to confirm these results.

References

1. Holly JM, Gunnell DJ and Smith DG: Growth hormone, IGF-I and cancer. Less intervention to avoid cancer? More intervention to prevent cancer? J Endocrinol 162: 321-330, 1999.
2. Ma P, Pollak MN, Giovannucci E, Chan JM, Tao Y, Hennekens CH and Stampfer MJ: Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J Natl Cancer Inst 91: 620-625, 1999.
3. Burroughs KD, Dunn SE, Barrett JC and Taylor JA: Insulin-like growth factor-I: a key regulator of human cancer risk? J Natl Cancer Inst 91: 579-581, 1999.
4. Ross JA, Perentesis JP, Robison LL and Davies SM: Big babies and infant leukemia: a role for insulin-like growth factor-1? Cancer Causes Control 7: 553-559, 1996.
5. Kim SY, Toretsky JA, Scher D and Helman LJ: The role of IGF-1R in pediatric malignancies. Oncologist 14: 83-91, 2009.
6. Laban C, Bustin SA and Jenkins PJ: The GH-IGF-I axis and breast cancer. Trends Endocrinol Metab 14: 28-34, 2002.
7. Bustin SA and Jenkins PJ: The growth hormone-insulin-like growth factor-I axis and colorectal cancer. Trends Mol Med 7: 447-454, 2001.
8. Smith GD, Gunnell DJ and Holly JM: Cancer and insulin-like growth factor-1: a potential mechanism linking the environment with cancer risk. BMJ 321: 847-848, 2000.
9. Jenkins PJ, Mukherjee A and Shalet SM: Does growth hormone cause cancer? Clin Endocrinol 64: 115-121, 2006.
10. Pollak MN, Schernhammer ES and Hankinson SE: Insulin-like growth factors and neoplasia. Nat Rev Cancer 4: 505-518, 2004.
11. Albanes D and Winick M: Are cell number and cell proliferation risk factors for cancer? J Natl Cancer Inst 80: 772-774, 1998.
12. Sanders M, Sorba S and Dainiak N: Insulin-like growth factors stimulate erythropoiesis in serum-substituted umbilical cord blood cultures. Exp Hematol 21: 25-30, 1999.
13. Williams GT and Smith CA: Molecular regulation of apoptosis: genetic controls on cell death. Cell 74: 777-779, 1993.
14. Petridou E, Dessypris N, Spanos E, Mantzoros C, Skalkidou A, Kalmanti M, Koliouskas D, Kosmidis H, Panagiotou JP, Piperopoulou F, Tzortzatou F and Trichopoulos D: Insulin-like growth factor-I and binding protein-3 in relation to childhood leukemia. Int J Cancer 80: 494-496, 1999.
15. Ritzen EM: Does growth hormone increase the risk of malignancies? Horm Res 39: 99-101, 1993.
16. McLaughlin CC, Baptiste MS, Schymura MJ, Nasca PC and Zdeb MS: Birth weight, maternal weight and childhood leukemia. Br J Cancer 94: 1738-1744, 2006.
17. Rangel M, Cypriano M, de Martino Lee ML, Luisi FA, Petrilli AS, Strufaldi MW and Franco MD: Leukemia, non-Hodgkin's lymphoma, and Wilms tumor in childhood: the role of birth weight. Eur J Pediatr 169: 875-881, 2010.
18. Gjerset RA, Yeargin J, Volkman SK, Vila V, Arya J and Haas M: Insulin-like growth factor-I supports proliferation of autocrine thymic lymphoma cells with a pre-T cell phenotype. J Immunol 145: 3497-3501, 1990.
19. Estrov Z, Meir R, Barak Y, Zaizov R and Zadik Z: Human growth hormone and insulin-like growth factor-1 enhance the proliferation of human leukemic blasts. J Clin Oncol 9: 394-399, 1991.
20. El-Badry OM, Romanus JA, Helman LJ, Cooper MJ, Rechler MM and Israel MA: Autonomous growth of a human neuroblastoma cell line is mediated by insulin-like growth factor II. J Clin Invest 84: 829-839, 1998.
21. El-Badry OM, Helman LJ, Chatten J, Steinberg SM, Evans AE and Israel MA: Insulin-like growth factor II-mediated proliferation of human neuroblastoma. J Clin Invest 87: 648-657, 1991.
22. Calle EE and Kaaks R: Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 4: 579-591, 2004.
23. Minniti CP, Tsokos M, Newton WA Jr and Helman LJ: Specific expression of insulin-like growth factor-II in rhabdomyosarcoma tumor cells. Am J Clin Pathol 101: 198-203, 1994.
24. Scotlandi K, Benini S, Sarti M, Serra M, Lollini PL, Maurici D, Picci P, Manara MC and Baldini N: Insulin-like growth factor I receptor-mediated circuit in Ewing's sarcoma/peripheral neuroectodermal tumor: a possible therapeutic target. Cancer Res 56: 4570-4574, 1996.
25. Yee D, Favoni RE, Lebovic GS, Lombana F, Powell DR, Reynolds CP and Rosen N: Insulin-like growth factor I expression by tumors of neuroectodermal origin with the t (11; 22) chromosomal translocation: a potential autocrine growth factor. J Clin Invest 86: 1806-1814, 1990.
26. Drummond IA, Madden SL, Rohwer-Nutter P, Bell GI, Sukhatme VP and Rauscher FJ III: Repression of the insulin-like growth factor II gene by the Wilms tumor suppressor WT1. Science 257: 674-677, 1992.
27. Werner H, Re GG, Drummond IA, Sukhatme VP, Rauscher FJ III, Sens DA, Garvin AJ, LeRoith D and Roberts CT Jr: Increased expression of the insulin-like growth factor I receptor gene, IGF1R, in Wilms tumor is correlated with modulation of IGF1R promoter activity by the WT1 Wilms tumor gene product. Proc Natl Acad Sci USA 90: 5828-5832, 1993.
28. Zumkeller W, Schwander J, Mitchell CD, Morrell DJ, Schofield PN and Preece MA: Insulin-like growth factor (IGF)-I, -II and IGF binding protein-2 (IGFBP-2) in the plasma of children with Wilms' tumor. Eur J Cancer 29A: 1973-1977, 1993.
29. Barozzi C, Ravaioli M, D'Errico A, Grazi GL, Poggioli G, Cavrini G, Mazziotti A and Grigioni WF: Relevance of biologic markers in colorectal carcinoma: a comparative study of a broad panel. Cancer 94: 647-657, 2002.
30. Hakam A, Yeatman TJ, Lu L, Mora L, Marcet G, Nicosia SV, Karl RC and Coppola D: Expression of insulin-like growth factor-1 receptor in human colorectal cancer. Hum Pathol 30: 1128-1133, 1999.