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Original Research Article

Detection of Tissue Factor Pathway Inhibitor-2 (TFPI-2) Gene Mutation in Sudanese Pediatric Patients with Leukemia

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ABSTRACT

Aim: we conducted our study to detect (TFPI-2) Gene Mutation in Sudanese Pediatric Patients with leukemia.
Materials & Methods: This study is a prospective analytical case control study was conducted to detect (TFPI-2) gene mutation in 98 Sudanese pediatric patients with leukemia; the population comprises all male and female pediatric patients, during the period of collection (2014-2015) admitted in Khartoum Radiation and Isotopes Centre (RIC), 97 individual pediatric (0 - 15 years) male and female apparently healthy from primary, Kindergartens and nurseries schools are selected as control group.
Results: Males comprised 52 (53.1%) while females are 46 (46.9%). The case group in our study was divided into three subgroups based on type of leukemia ALL, AML and CML. The frequency of ALL was 81 (82.7%), AML 12 (12.2%) and CML 5 (5.1%). TFPI-II gene detected in all samples, there were 14 (14.3%) in the patients group have mutation, 10 (71.4%) out of them were Homozygous and 4 (28.6 %) were Heterozygous. There is insignificant correlation between the patients which TFPI-II gene mutation have and other patients (p=0.635).
Conclusion: We concluded that ALL is the most common among Sudanese children and no strong association with TFPI-2 gene polymorphism and Sudanese pediatric patients with leukemia (p=0.635). Only 14.3% of Sudanese pediatric patients with leukemia have TFPI-II gene mutation.

Keywords: TFPI-2, mutation, pediatric, leukemia.

INTRODUCTION

Human tissue factor pathway inhibitor-2 (TFPI-2) is an extracellular matrix-associated Kunitz-type serine proteinase inhibitor that inhibits the plasmin- and trypsin-mediated activation of matrix metalloproteinases and inhibits tumor progression, invasion and metastasis. Previous studies have shown that TFPI-2 is down regulated in the progression of various tumors. [1]

Cancer in children less than 15 years old constituted about, 486 case 7% of the cancer cases recorded by the NCR. In Khartoum State, the most common cancer in children were leukemia, lymphoma, and cancer of the eye, bone, kidney, brain, breast, oral, liver, and stomach. Lymphomas were mostly non-Hodgkin's lymphoma and eye tumors were mostly retinoblastoma. [2]

Several studies approved that there strong relation between TFPI 2 and many types of cancers, includes glioma,

choriocarcinoma, pancreatic carcinoma, lung carcinoma, breast cancer, melanoma, lymphoma and leukemia through Epigenetic changes resulting in transcriptional silencing of cancer genes, Methylation, down regulation or over expression of TFPI-2. [3,4] No study has yet demonstrated the Tissue factor pathway inhibitor-2 (TFPI-2) gene mutation in Sudanese pediatric patients with leukaemia

MATERIALS AND METHODS

This study is a prospective analytical case control study was conducted to detect (TFPI-2) gene mutation in 98 Sudanese pediatric patients with leukemia; the population comprises all male and female pediatric patients, during the period of collection (2014-2015) admitted in Khartoum Radiation and Isotopes Centre (RIC), 97 individual pediatric (0-15 years) male and female apparently healthy from primary, Kindergartens and nurseries schools are selected as control group.

Inclusion criteria

Sudanese Individuals of both sex aged less than 15 years with leukaemia admitted in Khartoum Radiation and Isotopes Centre (RIC).

Exclusion Criteria

- Cured Patients.
- Patient with known history of coagulation disorder
- Inadequate, clot or hemolysed samples.

Methods

Blood collection and RNA extraction

Five ml venous blood was collected from each participant into EDTA tubes after consent obtained from each participant. Leukocytes will be prepared from peripheral blood samples after the addition of erythrocytes lysis buffer (150mM NH4Cl, 1mM KHCO3 and 0.1mM EDTA) (pH 7.3). Total RNA will be extracted from mononuclear cells by TRIzol reagent (Invetrogen, California. USA).

cDNA synthesis

For cDNA synthesis, 5µl of total RNA will be reversely transcribed in a reaction mixture containing (Reverse Transcriptase (RT) buffer: 20 mMTrisHCl, 50 mMKCl, pH 8.3; 5mM MgCl2, 10 mM DTT, 5mM random hexamers, 20 units RNAase inhibitor, 10 units RT enzyme, 1mM dNTP and H2O to a total volume of 20 µl) at 42°C for 60 minutes. RT enzyme will be denatured by incubating the reaction at 99°C for 5 minutes.

RT-PCR

Primers specific for the (TFPI-2) will be used to amplify the cDNA as follows A 450 bp fragment of the full-length TFPI-2 coding sequence was amplified from primers

5`-GGGGTACCG CTT TCT CGG ACG CCT TG-3` (for-ward) and 5`-CGG GAT CCT GAT TTG TTT CCTCAT GCT GTC-3` (reverse)

For the first round of nested PCR, 3µl of cDNA product will be amplified in a reaction mixture containing 0.2mM dNTP mix, 1.9µM MgCl2, 0.5u Taq polymerase, 1X PCR buffer, 0.5 mM of each primer and H2O to 25 µl. the PCR cycling condition will be 94°C for 30s, 64°C for 60s, 72 °C for 60s for 35 cycle and 72°C for 10 minutes final extension .For the second round of nested PCR, 1µl aliquot of the first round of nested PCR product will be amplified with specific primers using the same PCR reaction mixture and the PCR cycling condition of the first round of nested PCR.

Restriction fragment length polymorphism (RFLP) analysis of the TFPI-II

PCR was carried out according to the above protocols, using mismatched primers as shown. Having checked PCR products by running 5ul on 1% agarose gel, they were incubated over night with 1.5 U of Mae III (Boehringer Mannheim) at 55 oC in a total volume of 20ul. After loading the samples on 2.5% agarose gel, products were analyzed under UV-light. The designed

mismatched primer creates a cutting site at the position of GTNAC which can be recognized by Mae III endonuclease. The normal TFPI-II (237 bp fragment) was cut in to three smaller fragments of 145, 64 and 26 bp. In the presence of mutation (G:A transition in exon the fragment was cut into two smaller fragments of 171 and 64 bp owing to the absence of the GTNAC cutting site in the mutant TFPII . Finally, in the case of heterozygous DNA, There were four fragments of 171, 145, 64 and 26 bp.

Data analysis

Data were entered and analyzed by SPSS programme (version: 17.0). All demographic data of the study population were presented as mean and SD in the text and P. value was used for detecting the power of relationship between the determinant and the outcome and 95% confidence interval was calculated Data were analyzed using the Chi-square test for comparison the prevalence of TFPI-2 gene mutation between patients and controls (The test considered significant when P. value<0.05)

Ethical clearance

Before collection of data and samples from humans, must be need to obtain Research Ethics Approval from the Ethics Committee before starting the research.

All patients were requested verbally and had completed an informed consent.

RESULTS

The participants included 200 Children aged 0 - 15 years. 100 Out of them, was already diagnosed with leukemia and 100 healthy Children as control. Two samples are excluded from patient's samples and 3 samples from control samples because either of insufficiency or clotted samples.

Males comprised 52(53.1%) while females are 46(46.9%) with mean age for all 9.03 years and SD 4.058 were in the control group Gender wise males are 53 (54.6%) and females are 53 (45.4%) with mean age for all 8.08 years and SD 4.033

The case group in our study was divided into three subgroups based on type of leukemia ALL, AML and CML. The frequency of ALL was 81 (82.7%), AML 12 (12.2%) and CML 5 (5.1%). (Table 2) TFPI-II gene detected in all samples, there were 14 (14.3%) in the patients group have mutation, 10 (71.4 %) out of them were Homozygous and 4 (28.6 %) were Heterozygous. No participant among control group (0%) had TFPI-II gene mutation. (Table 3) There is insignificant correlation between the patients which TFPI-II gene mutation have and other patients (p=0.635). (Table 5)

Table (1): shows gender distribution in case and control group

Sex		Frequency	Percent
Test group	Male	52	53.1 %
	Female	46	46.9 %
	Total	**98**	**100 %**
Control group	Male	53	54.6 %
	Female	53	45.4 %
	Total	**97**	**100 %**

Table (2): shows type Frequency of leukemia in the Case group

Case group	Frequency	Percent
ALL	81	82.7 %
AML	12	12.2 %
CML	5	5.1 %
Total	**98**	**100 %**

Table (3): Shows Frequency& Percent of TFPI-II gene mutation in the Case & Control groups

TFPI-II gene	Case		Control	
	Frequency	Percent	Frequency	Percent
Without mutation	84	85.7 %	97	100%
With Mutation	14	14.3 %	0	0 %
Total	**98**	**100 %**	**97**	**100 %**

Table (4): Shows Frequency and Percentage of TFPI-2 gene mutation type

TFPI-2 gene mutation	Frequency	Percent
Homozygous mutation	10	71.4 %
Heterozygous mutation	4	28.6 %
Total	**14**	**100 %**

Table (5): Shows TFPI-2 gene mutation significance in the patient group

Case group	Normal Patient	Patient with Mutation	Total	Sig.
ALL	69	12	81	0.635
AML	10	2	12	
CML	5	0	5	
Total	**84**	**14**	**94**	

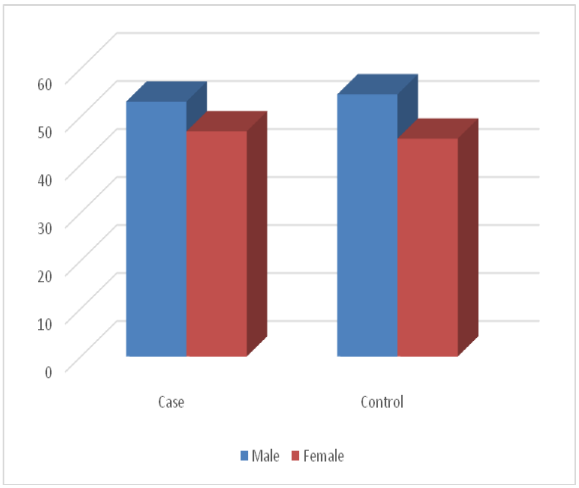

Figure (1) Shows gender distribution in case and control group

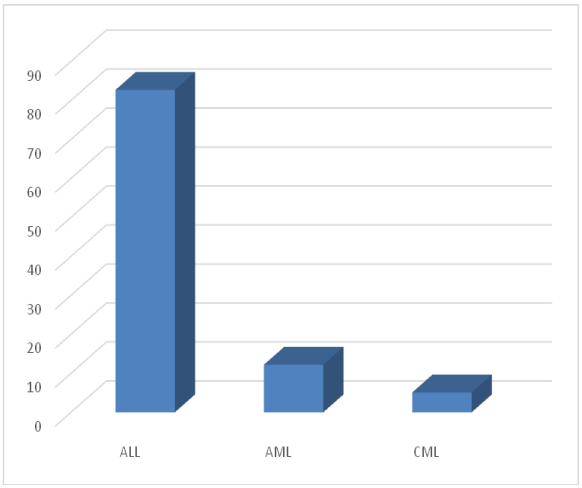

Figure (3) Shows types of leukemia in patient group

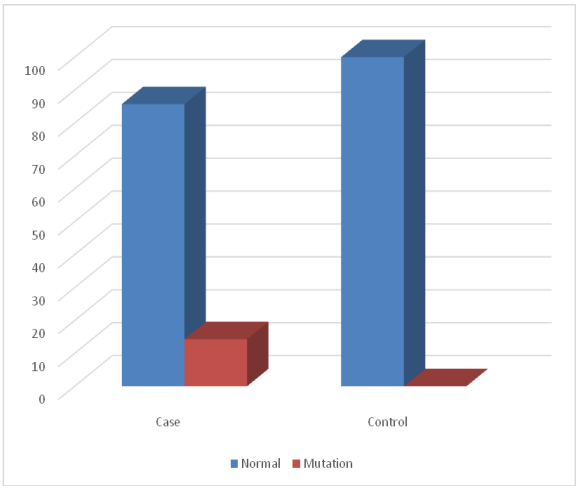

Figure (2) ShowsTFPI-2 gene mutation by RFLP in the case and control group

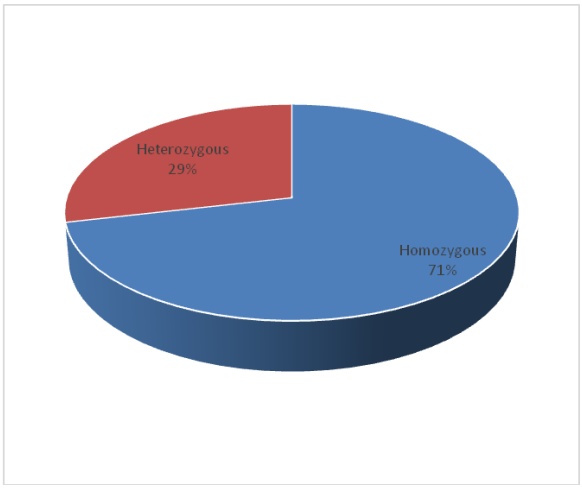

Figure (4) Shows TFPI-2 gene mutation Percentage

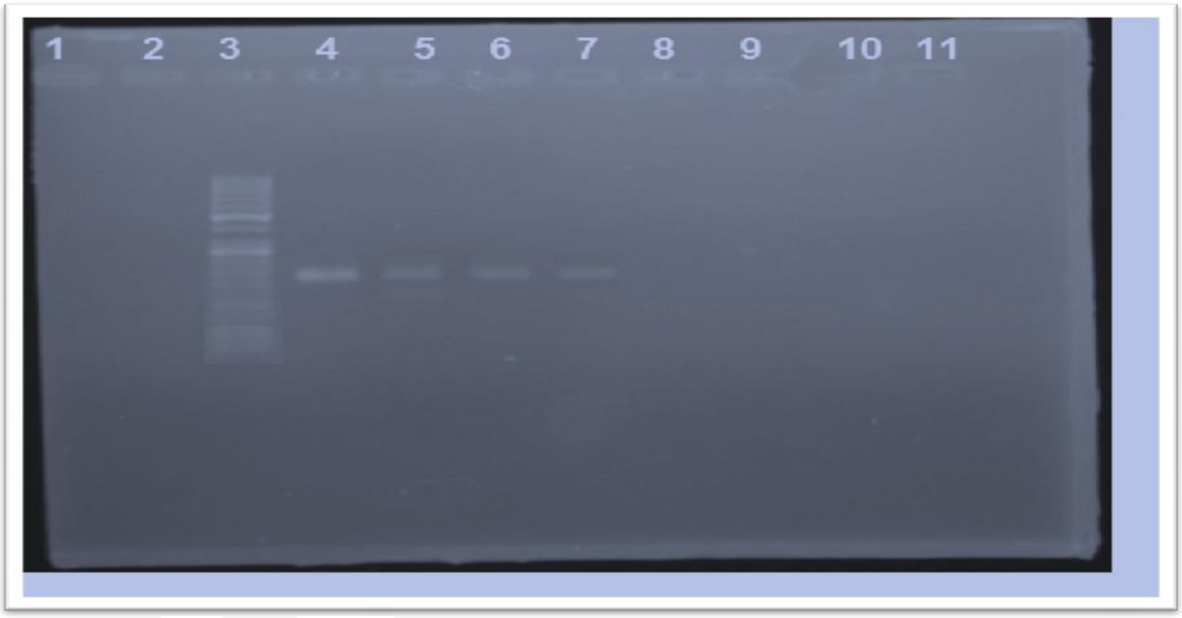

Fig (5) Control Result for TFPI-II restriction fragment length polymorphism (RFLP) analysis:
1 - Negative Control. 2 - TFPII Negative Sample., 3 - 100pb DNA Marker., 4- 4 To 7- positive sample for TFPII., 5- 8 To 11 - negative sample for TFPII.

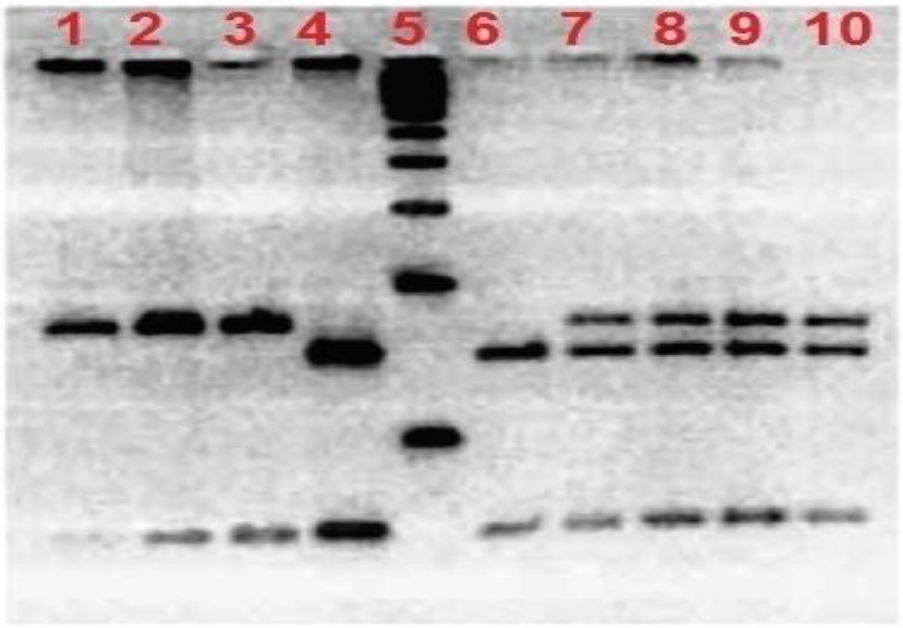

Figure (6) Patient Result for TFPI-II restriction fragment length polymorphism (RFLP) analysis

Restriction fragment length analysis of the TFPII polymorphism using Mae III (lanes numbered from left to right). The normal TFPII is digested into three fragments of 145, 64 and 26 bp (lanes 4, 6). Presence of homozygous TFPII polymorphism gives rise to two fragments of 171 and 64 bp (lanes 1±3), and in the case of heterozygous DNA, there are 4 fragments of 171, 145, 64 and 26 bp (lanes 7±10).

DISCUSSION

Some studies proved that TFPI-2 might be considering as a putative tumor suppressor gene in nasopharyngeal carcinoma NPC [5] and other studies concluded that TFPI-2 promoter methylation might be considered as prognostic marker in glioblastoma. [6]

And other study stated that a possibility of TFPI-2 may have therapeutic value in the treatment of malignant esophageal carcinoma, [7] and also methylation-specific polymerase chain reaction (qMSP) for TFPI 2 could be used as a marker in cancer screening method. [8]

All these studies focusing in epigenetic changes to TFPI2 in patient with malignancy and proved that there is strong correlation between them, but in the level of gene mutation there is one study which stated that there is one apparent linkage, disequilibrium among single nucleotide polymorphisms (SNPs) of TFPI-2 gene in coronary heart disease CHD patients. [9]

In our study we detect Tissue Factor Pathway Inhibitor-2 (TFPI-2) Gene Mutation in Sudanese Pediatric Patients with leukemia. The frequency of ALL was 81 (82.7%), AML 12 (12.2%) and CML 5 (5.1%), this was supported by a study done by Coebergh, *et al.* 2006, [10] who reported from the Automated Childhood Cancer Information System project, that in Europe, acute lymphoblastic leukaemia (ALL) accounts for around 80% of leukaemia among children aged 0 - 14 years. There is no significant different between case and control group in the age (P value 0.1027).

Reverse transcriptase polymerase chain reaction followed by Restriction fragment length polymorphism /RFLP) electrophoresis analysis for the TFPI-II was done.

TFPI-II gene detected in all samples, the prevalence of TFPI-2 gene mutation was tested and calculated in both case and control groups. The presence of TFPI-2 gene mutation was higher among cases group compared to the controls group. The prevalence of the mutation among case

group was 14 (14.3%) of the patients 10 of them Homozygous and 4 were Heterozygous. No participant (0%) has TFPI-II gene mutation among control group.

We did not found a strong association with TFPI-2 gene polymorphism and Sudanese pediatric patients with leukemia, there is insignificant correlation between the patients with TFPI-II gene mutation (p=0.635) and other patients.

TFPI-2 gene located on long arm of chromosome 7 (7q22), our result may be due to complete or partial deletion of chromosome 7, this justification agree with Heerema, *et al*. 2004 [11] who examined Complete or partial loss of chromosome 7, predominantly monosomy 7 or deletions of 7q, is associated with a variety of myeloid disorders, including acute myelogenous leukemia (AML) in children. It has been hypothesized that there is a tumor suppressor gene (TSG) on chromosome arm 7q that contributes to the pathogenesis of these diseases, and several chromosomal bands have been identified that contain commonly deleted segments, including 7q22 regions, he examined the frequency and prognostic significance of losses and deletions of chromosome 7 in a large cohort studied of children with ALL. Loss of all or part of chromosome 7 was found in 75 of 1880 (4%) patients.

Losses involving chromosome 7 are relatively frequent in childhood ALL. Deletions of 7p confer an inferior outcome in children with ALL, regardless of the presence of other poor prognostic features, whereas deletions of 7q are not associated with a worse outcome.

Our justification also supported by. Saito, *et al*. 2005, [12] and Sell, *et al*. 2004 [13] who conclude that Several highly aggressive cancers delete the locus for the TFPI-2 gene in chromosome 7q region, which results in a complete lack of TFPI-2 protein expression in these cells.

Russo C, et al. 1991 [14] found in 57 Philadelphia chromosome-positive (Ph1) ALL cases (23%) were also found to have partial or complete monosomy 7 (-7). This subgroup of children with Ph1/-7 ALL was comprised primarily of older males with early B-lineage ALL.

Dong 2001 [15] stated in his study that TFPI-2 transcription could be regulated by polymorphisms in the promoter sequence affecting transcription factor binding sites and absence of TFPI-2 expression can also occur through deletion of the chromosomal region 7q22, as in prostate cancer.

CONCLUSION

We concluded that ALL is the most common among Sudanese children and no strong association with TFPI-2 gene polymorphism and Sudanese pediatric patients with leukemia (p=0.635). Only 14.3% of Sudanese pediatric patients with leukemia have TFPI-II gene mutation.

REFERENCES

1. Yong Xui, Xliaolin Qin, Jinjimg Zhou, Zhiguang TU, Xiao Bo, Wuxain LI, Xiaoqing Fan and Y Zhang. Tissue factor pathway inhibitor-2 inhibits the growth and invasion of hepatocellular carcinoma cells and is inactivated in human hepatocellular carcinoma. Oncology Letters. 2011; 2 (5): 779-783
2. Intisar E Saeed, Hsin-Yi Weng, Kamal H Mohamed, and Sulma I Mohammed (2014).Cancer Med. Cancer incidence in Khartoum, Sudan: first results from the Cancer Registry, 2009-.Cancer Med. 2014; 3(4): 1075-1084.
3. Hamamoto J, Soejima K, Naoki K, Yasuda H, Hayashi Y, Yoda S, Nakayama S, Satomi R, Terai H, Ikemura S, Sato T, Arai D, Ishioka K, Ohgino K, Betsuyaku TM ethylation-induced down regulation of TFPI-2 causes TMPRSS4 over expression and contributes to on cogenesis in a subset of non-small-cell lung carcinoma. Cancer Sci. 2015; 106(1):34-42.
4. Prakasha Kempaiah, Hitendra S Chand and Walter Kisiel. Molecular Cancer. Identification of a human TFPI-2 splice variant that is upregulated in human tumor tissues Kempaiah. 2007; 20:6.
5. Shumin Wang, Xue Xiao, Xiaoying Zhou, Tingting Huang, Chunping Du, Nana Yu, Yingxi Mo, Longde Lin,

Jinyan Zhang, Ning Ma, Mariko Murata, Guangwu Huang and Zhe Zhang. TFPI-2 is a putative tumor suppressor gene frequently inactivated by promoter hypermethylation in nasopharyngeal carcinoma. B.M.C Cancer. 2010; 10:617.

6. Paulina Vaitkienė, Daina Skiriutė, Kęstutis Skauminas, Arimantas Tamašauskas Medicina (Kaunas). Associations between TFPI-2 Methylation and Poor Prognosis in Glioblastomas. Neuroscience Institute, Medical Academy, Lithuanian University of Health Sciences. 2012; 48(7): 345-9.
7. Ran Y, Pan J, Hu H, Zhou Z, Sun L, Peng L, Yu L, Sun L, Liu J, Yang Z. Human gene therapy. A novel role for tissue factor pathway inhibitor-2 in the therapy of human esophageal carcinoma 2009; 20(1):41-9.
8. Hibi K, Goto T, Shirahata A, Saito M, Kigawa G, Nemoto H, SanadaY. Detection of TFPI2 Methylation in the Serum of Gastric Cancer Patients. Anticancer research Yokohama. 2011; 227-8501.
9. Liu Rong-Le , LI Jian , Ni Huan-chun, Jin Bo, Zhu Jun, Li Yan-lin, Shen Wei, Shi Hai-Ming, MA Duan , LuoXin-Ping. Clinical study of the association between tissue factor pathway inhibitor 2 (TFPI-2) gene polymorphisms and acute coronary syndrome. Fudan University Journal of medical science. 2013; 40 (6): 685.
10. Coebergh J-W et al. Leukaemia incidence and survival in children and adolescents in Europe during 1978-1997. Report from the Automated Childhood Cancer Information System project. European Journal of Cancer. 2006; 42:2019-2036.
11. Heerema, J B Nachman, H N Sather, M K La, R Hutchinson4, B J Lange, B Bostrom, P G Steinherz, P S Gaynon and F M Uckun. Deletion of 7p or monosomy 7 in pediatric acute lymphoblastic leukemia is an adverse prognostic factor: a report from the Children's Cancer Group. Leukemia. 2004; 18: 939-947.
12. Saito E, Okamoto A, Saito M, et al. Genes associated with the genesis of leiomyoma of the uterus in a commonly deleted chromosomal region at 7q22. Oncol Rep. 2005; 13:2386-2397.
13. Sell SM, Tullis C, Stracner D, Song CY, Gewin J. Minimal interval defined on 7q in uterine leiomyoma. Cancer genetics. 2005; 157(1): 67-69.
14. C Russo, A Carroll, S Kohler, M Borowitz, M Amylon, A Homans, A Kedar, J Shuster, V Land, W Crist. Philadelphia chromosome and monosomy 7 in childhood acute lymphoblastic leukemia: a Pediatric Oncology Group study. Bood.1991; 77(5): 1050-1056.
15. J.T. Dong. Chromosomal deletions and tumor suppressor genes in prostate cancer, Cancer and metastasis reviews. 2001; 20 (3-4): 173-193.

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