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DETECTION OF GENOMIC MARKERS BY RANDOM AMPLIFIED POLYMORPHIC DNA ANALYSIS IN LEUKEMIA PATIENTS

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Abstract:

One of the hematological malignancies brought on by the abnormal proliferation of immature leukocytes is leukemia. Numerous epigenetic and genetic changes in hematopoietic progenitor cells are the cause of it. The Random Amplification of Polymorphic DNA (RAPD) technique has been utilized in the presented work in order to assess DNA polymorphisms and discover genomic markers in diverse leukemia types. The analysis utilizing a random primer regarding decamer oligonucleotides OPJ-04 produced distinct profiles of amplified fragments of DNA in the genomic DNA of 3 different types of the leukemia patients. The unique band (2178 bp), which is present in 60 and 66.6% of chronic myeloid leukemia (CML) and acute myeloid leukemia (AML), respectively, exhibited a significant difference ($P \leq 0.01$). While detected in only 20% of cases chronic lymphoid leukemia (CLL), and absent in the remaining leukemia patients and healthy controls. The results showed that the distinct bands (1737 and 1513 bp), which were present in all leukemia patients, however absent in healthy controls, showed extremely significant differences ($P \leq 0.01$). Similarly, results have revealed highly significant differences ($P \leq 0.010$) in fourth distinct band (1202 bp) that detected in all AML, 86.6% of CML, and 90% of CLL, and lacking in the remaining leukemia patients and in all healthy controls The results also showed that there have been significant differences ($P \leq 0.01$) in the amplified band (750 bp), which was absent in 73.3% of CML patients, but present in the remaining CML patients, along with all AML patients, CLL patients, and healthy controls. The 4 amplified DNA fragments (1031, 870, 600, and 300 bp) that detected in all healthy controls and leukemia patients, at the same time, showed no significant differences in the results. The recognized DNA polymorphisms through the arbitrary primer OPJ-04 may be used as a genetic marker to design a successful RAPD primer for the diagnosis of leukemia patients.
Keywords: *Genetic Markers, DNA Polymorphism, Leukemia, RAPD-PCR* .

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Introduction:

Leukemia is characterized by the neoplastic growth of leukocyte precursors in the bone marrow. Leukemia's main types are acute and chronic; each major type is further subdivided into lymphoid and myeloid (Sánchez-Aguilera and Méndez-Ferrer, 2017). Acute leukemias are typically aggressive diseases that cause accumulation of haemopoietic progenitors blast cells in bone marrow. Majorly, such diseases result in bone marrow failure due to accumulation of blast cells, even though tissue infiltration may also occur as well. Compared with acute leukemia, chronic leukemia progresses slower and the neoplastic cells are more mature (Sweet and Asghari, 2021). Leukemia types are Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL), Chronic Myeloid Leukemia (CML), and Chronic lymphoid Leukemia (CLL) (Dong *et al.*, 2020). Leukemia incidence differs by sex and age. These discrepancies are mostly related to exposure to environmental and genetic risk factors (Boonhat and Lin, 2020). In Iraq, leukemia was the fifth most common form of cancer, the incidence is strongly related to age, with maximum rates of incidence being in the old individuals, it affects males more than females (Iraqi Cancer Board, 2020). Several studies demonstrated that leukemia initiation and progression is a result of the accumulation of genetic abnormalities and to the epigenetic changes. Genetic abnormalities include gene mutations like (deletion, duplication or insertion) and chromosomal anomalies such as (translocations, inversion or deletion). The RAPD-PCR approach was employed to determine the genetic abnormalities in human malignancies, and it was discovered that various types of cancers commonly exhibited genetic alterations, for instance renal cancer (El-Far *et al.* 2013), breast cancer (Allami and Dragh, 2022; Ismaeel, 2013; Papadopoulos *etal.*, 2002), colon cancer (Azeez *etal.*, 2016), oral squamous cell carcinoma (Yass and Sabah, 2012), bladder cancer (El-Far *etal.*, 2013), and carcinoma of hepatocellular (Zhang *etal.*, 2004; Xian *etal.*, 2005). Other aspects of cancer research have also been conducted using RAPD-PCR analyses like, carcinogensis and genotoxicity studies (Jha & Atienzar 2006), DNA damage and mutation detection (Atienzar *etal.*, 2002);, and Identifying novel DNA amplifications (Garnis *etal.*, 2004) in leukemia (Saleh *etal,* 2010: Odero *etal.*, 2001) and in lymphoma (Scarpa *etal.*, 2001).

Materials and Methods

Collection of Blood Samples and Extraction of DNA:

This study included forty patients diagnosed with leukemia of different types (AML, CML, and CLL), as well as fifteen healthy controls of similar ages and gender. Three-milliliters of blood samples were withdrawn from each case subjects and distributed into tubes containing anticoagulant (K3EDTA) for the genomic DNA extraction. In addition, gDNA has been obtained from the blood sample for both patients and healthy subjects and used for molecular study by gDNA purification kit (RIBO-Sorb-AM / AmpliSens) as fellow by manufacture's protocol.

DNA Amplification and Gel Electrophoresis.

The genomic DNA of both patients and healthy subjects were amplified through PCR technique, using arbitrary primer OPJ-04 with sequence (5⁻ CCGAACACGG 3⁻). The PCR reaction mixtures were brought together according to the manufacture procedure of the master mix (AccuPower® PCR PreMix, Bioneer). The 40 cycles of 94°C for 30sec., 37°C for 1min., and 72°C for 2min. that make up the thermal reaction conditions. The PCR products that had been amplified were electrophoresed in 1.20% agarose gel with 1X TBE buffer. One hour of 70 v electrophoresis was performed with the use of a UV-light transillminator, DNA

bands have been seen under UV light. The molecular size of amplified fragments was estimated based on standard curve that represents the correlation between molecular band size of a DNA ladder that has been measured by the base pairs and the migration distance. A DNA ladder with molecular size ranging from 1031 to 100 bp was used in this study.

Molecular size of amplified fragments have been estimated according to standard curve that represents relationship between the molecular band size of ladder measured by the base pairs and the migration distance. DNA ladder was used this study ranged from 1031 to 100 bp.

Statistical Analyses:

An association between bands that are monomorphic, polymorphic, or unique and RAPD-PCR primer for healthy controls and leukemia patients was discovered using the SAS (2018) program. In the presented work, a significant comparison between percentage (0.050 and 0.010 probability) was made with the use the Chi-square test.

Results and Discussion

This work included molecular genetic analysis of forty leukemia patients distributed into three groups: (15) AML patients, (15) CML patients, and (10) CLL patients. The age of the patients was in range of 21 and 70 years old, with higher incidence in age group (41 – 50) years and incidence in the males is higher than in the females as demonstrated in table (1). Leukemia development has been partly ascribed to the genetic risk factors, like initiation of the chromosomal aberrations. Moreover, exposure to chemicals, pesticides, radiation, and other environmental factors was associated with an increased risk of leukemia (Dong et al., 2020). RAPD-PCR analysis with an arbitrary primer regarding decamer oligonucleotides was performed using gDNA isolated from forty leukemia patients and fifteen healthy individuals as a control. A primer used in this study revealed distinct bands with distinctive patterns. In addition, primer OPJ-04 produced nine types of the amplified DNA fragments derived from the gDNA that had been extracted from normal individuals and three types of leukemia patients. The molecular weights of amplified DNA fragments have been 2187, 1737, 1513, 1202, 1031, 870, 750, 600 and 300 bp. Four polymorphic amplified bands obtained by primer OPJ-04 with moderate intensities appeared in the gDNA of all types of leukemia patients and not obtained in normal individuals (figures 1, 2, and 3). The molecular sizes regarding such amplified DNA fragments have been 2187, 1737, 1513, and 1202 bp. The largest amplified DNA fragment with molecular size 2178 bp detected in 66.6% of AML, 60% of CML, and 20% of CLL, and missing in the remaining leukemia patients and in all healthy controls as seen in Fig. 1, Fig 2, and Fig 3. The results exhibited a significant difference ($P \leq 0.01$) in this unique band between leukemia patients and healthy controls (tables 2, 3, and 4). The second amplified band with molecular weight (1737bp.) was present in all leukemia patients, but lacking in all healthy controls as shown in figures (1, 2, and 3). The results in tables (2, 3, and 4) demonstrated highly significant differences ($P \leq 0.01$) in this unique band between leukemia patients and healthy controls. In addition, the presented work indicated a highly significant differences ($P \leq 0.01$) in amplified band (1513 bp), which present in all AML and CLL patients, and in 60% of CML but not detected in all healthy controls (tables 2, 3, and 4). Similarly, results have shown highly significant differences ($P \leq 0.01$) in fourth distinct band (1202 bp) that detected in all AML, 86.6% of CML, and 90% of CLL, and lacking in the remaining leukemia patients and in all healthy controls. Moreover, the results presented in tables (2, 3, and 4) showed highly significant differences ($P \leq 0.01$) in amplified band (750 bp) which was absent in 73.3% of chronic myeloid leukemia patients but present in the remaining chronic myeloid leukemia patients, as well as all acute myeloid

leukemia patients, chronic lymphoid leukemia patients, and all healthy controls. On the other hand, the results revealed no significant differences in the four amplified DNA fragments (1031, 870, 600, and 300 bp) which appeared in all leukemia patients and healthy controls. However, it has been possible to observe increase in relative intensities of monomorphic bands with molecular size 870 and 600bp in leukemia patients than in control individuals as seen in figures (1, 2, and 3).

The RAPD method offers a rapid, simple, and effective way of screening the whole genome to detect genetic instability. It also identifies DNA markers without specific information regarding DNA sequences. Our study showed that DNA polymorphisms are more frequent in leukemia patients' gDNA than in normal individuals' gDNA. This was done using primer J-04 which demonstrated that leukemia patients showed newly amplified DNA fragments and missing bands compared to healthy controls. Leukemia patients suffering from different mutations will impact primer-template binding sites. Various types of gene mutations and chromosomal abnormalities like deletion, duplication, substitution, and insertion. These results in a change in the nucleotide sequence in the gDNA of leukemia patients compared to normal controls. Consequently, they have different numbers of primer binding sites in their genomic DNA. So the number of amplified bands varies due to gain or missing bands. Multiple investigators have reported similar genomic changes in various types of cancer, like detection of new band in breast cancer in comparison to control individuals by Ismael (2013). Similarly, El-Far et al. (2013) indicated genomic alterations in bladder cancer and renal cancer. Additionally, genomic instability has been reported in colon cancer (Azeez, 2016), and oral squamous cell carcinoma (Yass and Sabah, 2012). Moreover,in compared to healthy controls, there was an increase within the level of intensity of the bands identified in the RAPD analysis of gDNA from leukemia patients. In that regard, alterations in the intensity of the amplified bands reported by other researchers (Xian *etal.*, 2005; Papadopoulos *etal.*, 2002). The present work demonstrated newly added DNA fragments of 1737bp and 1513bp in the gDNA of CML, AML and CLL when using J-04 primer. These findings may lead to the assumption of the use of these DNA fragments as molecular marker for fast, simple, and easy prognosis and diagnosis of leukemia with reducing coast and time. Moreover, further research should be conducted with more leukemia patients and healthy controls to verify the findings of this research.

Conclusion:

Leukemia patients' gDNA showed signs of genetic instability, which identified through RAPD primers like primer OPJ-04. It provided a distinct band in all leukemia patients when put to comparison with healthy people, and it might be exploited in the future to create molecular markers for the prognosis and diagnosis of CML, AML, and CLL patients.

Table (1): Distribution of leukemia patients by age and gender

Age (Years)	Gender		Total	(%)
	Female	Male		
21 – 30	4	4	8	20
31 – 40	4	5	9	22.5
41 – 50	5	5	10	25
51 – 60	2	2	4	10
61 – 70	3	6	9	22.5
Total	18	22	40	100

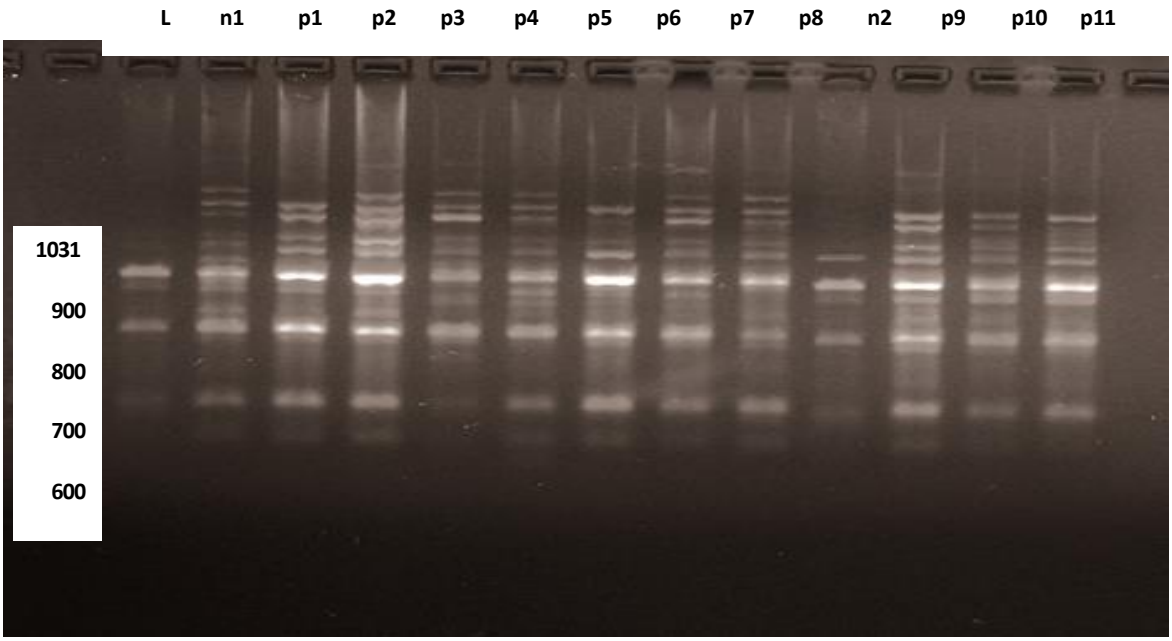

Figure (1): RAPD-PCR patterns of AML patients obtained with OPJ-04 primer. N1 and n2 indicate normal controls. P1, p2, p3, p4,p5, p6, p6, p7, p8, p9, p10, and p11 indicate the AML patients,. L represents DNA ladder in bp.

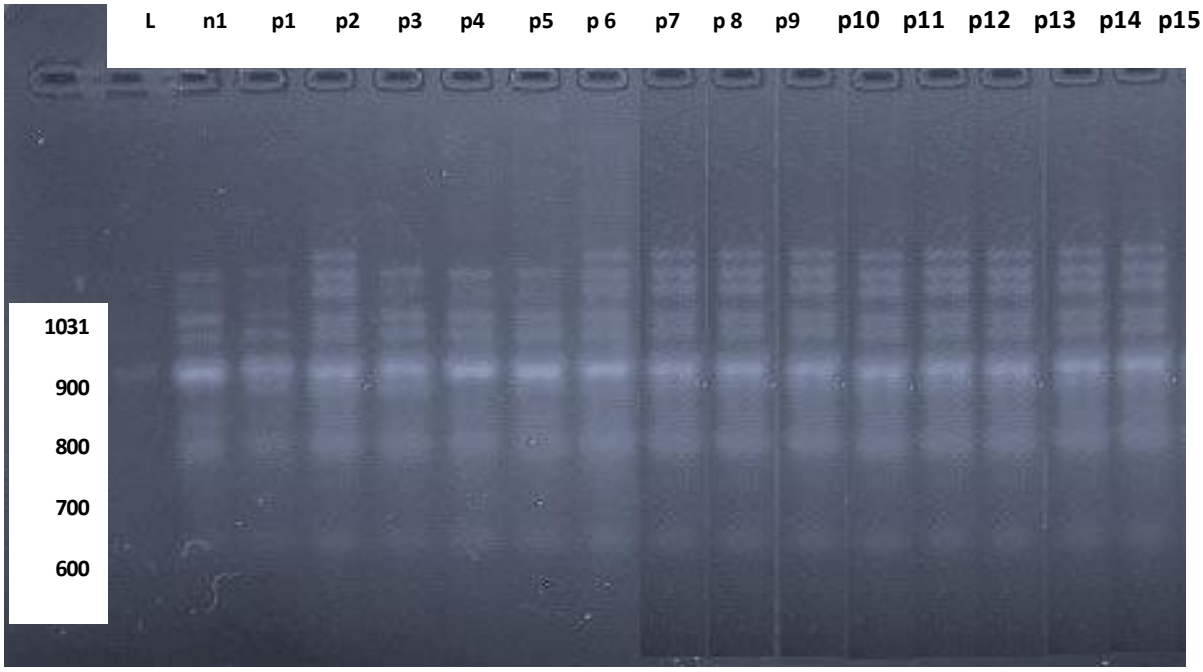

Figure (2): RAPD-PCR patterns of CML patients obtained with OPJ-04 primer. N1 indicate normal control. P1, p2, p3, p4,p5, p6, p6, p7, p8, p9, p10, p12, p13, p14, and p15 indicate the CML patients,. L represents DNA ladder in bp.

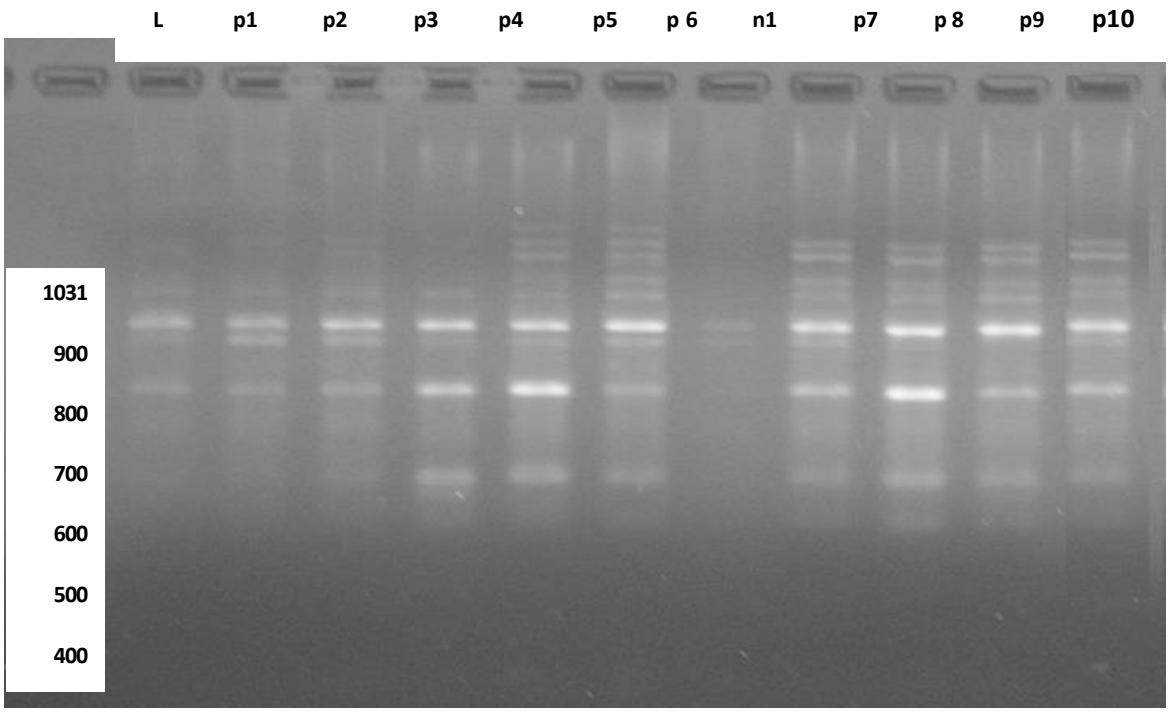

Figure (3): RAPD-PCR patterns of CLL patients obtained with OPJ-04 primer. N1 indicate normal control. P1, p2, p3, p4,p5, p6, p6, p7, p8, p9, and p10 indicate the CLL patients,. L represents DNA ladder in bp.

Table 2: Comparison of RAPD-PCR Patterns of Amplified Detected with Primers OPJ-04 Between AML Patients and Normal Controls

	Normal Controls				AML Patients				
	Amplified band		No amplified band		Amplified band		No amplified band		
M.Wt bp	No.	%	No.	%	No.	%	No.	%	P-value
2187	0	0	15	100	10	66.6	5	33.3	0.0004 **
1737	0	0	15	100	15	100	0	0	0.0001 **
1513	0	0	15	100	15	100	0	0	0.0001 **
1202	0	0	15	100	15	100	0	0	0.0001 **
1031	15	100	0	0	15	100	0	0	1.00 NS
870	15	100	0	0	15	100	0	0	1.00 NS
750	15	100	0	0	15	100	0	0	1.00 NS
600	15	100	0	0	15	100	0	0	1.00 NS
300	15	100	0	0	15	100	0	0	1.00 NS
P-value	---	0.0001 **	---	0.0001 **		0.0376 *		0.0376 *	---

***: represents a significant difference (P≤0.05), **: highly significant (P≤0.01), and NS: non-significant.**

Table 3: Comparison of RAPD-PCR patterns of Detected with Primers OPJ-04 Between CML Patients and Normal Controls.

	Normal Controls				CML Patients				
	Amplified band		No amplified band		Amplified band		No amplified band		
M.Wt bp	No.	%	No.	%	No.	%	No.	%	P-value
2187	0	0	15	100	9	60	6	40	0.0081 **
1737	0	0	15	100	15	100	0	0	0.0001 **
1513	0	0	15	100	9	60	6	40	0.0004 **
1202	0	0	15	100	13	86.6	2	13.3	0.0001 **
1031	15	100	0	0	15	100	0	0	1.00 NS
870	15	100	0	0	15	100	0	0	1.00 NS
750	15	100	0	0	4	33.3	11	73.3	0.0002 **
600	15	100	0	0	15	100	0	0	1.00 NS
300	15	100	0	0	15	100	0	0	1.00 NS
P-value	---	0.0001 **	---	0.0001 **		0.0001 **		0.0001 **	---

***: represents a significant difference (P≤0.05), **: highly significant difference (P≤0.01), and NS: non-significant.**

Table 4: Comparison of RAPD-PCR patterns Detected with Primers OPJ-04 Between CLL Patients and Normal Controls.

	Normal Control				CLL Patients				
	Amplified band		No amplified band		Amplified band		No amplified band		
M.Wt bp	No.	%	No.	%	No.	%	No.	%	P-value
2187	0	0	10	100	2	20	8	80	0.049 *
1737	0	0	10	100	10	100	0	0	0.0001 **
1513	0	0	15	100	10	100	0	0	0.0001 **
1202	0	0	15	100	9	90	1	10	0.0001 **
1031	10	100	0	0	10	100	0	0	1.00 NS
870	10	100	0	0	10	100	0	0	1.00 NS
750	10	100	0	0	10	100	0	0	1.00 NS
600	10	100	0	0	10	100	0	0	1.00 NS
300	10	100	0	0	10	100	0	0	1.00 NS
P-value	---	0.0001 **	---	0.0001 **		0.0001 **		0.0001 **	----

*: represents a significant difference (P≤0.05), **: highly significant difference (P≤0.01), and NS: non-significant.

References

Allami, Z. Z. G., and Dragh, M. A. (2022). Identification of Some Breast Cancer Related Genes by RAPD Technique in Maysan Province, Iraq. Revis Bionatura, 7 (1), 20-28.

Atienzar, F. A., and Jha, A. N. (2006). The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. Mutation Research/Reviews in Mutation Research, 613(2-3), 76-102.

Atienzar, F. A., Venier, P., Jha, A. N., and Depledge, M. H. (2002). Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 521(1-2), 151-163.

Azeez, N. A., Alshamaa, S. D. S., and Hadiy, I. A. (2016). Used OPA-13 And OPA-07 Reptitative Sequence For Detection Genomic Instability In Early Stage Colon Cancer By RAPD analysis.

Boonhat, H., and Lin, R. T. (2020). Association between leukemia incidence and mortality and residential petrochemical exposure: A systematic review and meta-analysis. Environment International, 145, 106090.

Dong, Y., Shi, O., Zeng, Q. Lu, X., Wang, W., Li, Y. and Wang, Q. (2020). Leukemia incidence trends at the global, regional, and national level between 1990 and 2017. Exp Hematol Oncol 9, 14.

El-Far M, Abol-Enein H, Zakaria A, and El-Gedamy M (2013) Detection of Genomic instability in renal cancer by random amplified polymorphic DNA analysis from urine sample as a non-invasive method: potential use in diagnosis. Cancer Sci Res. 1(1): 7.

El-Far, M., Abol-Enein, H., Zakaria, A., and El-Gedamy, M. (2013). Application of random amplified polymorphic DNA-PCR technique in early diagnosis of bladder cancer using urine samples. Current Topics in Bio. Res. 15 (2) 23-34.

Garnis, C., Coe, B.P., Ishkanian, A.,Zhang, L., Rosin, M.P., and Lam, W.L. (2004). Novel regions of amplification on 8q distinct from the MYC locus and frequently altered in oral dysplasia and cancer. Genes, Chromosomes Cancer, 39: 93–98.

Iraqi Cancer Board. (2020). Results of Iraqi Cancer Registry 2018. Ministry of Health. Baghdad-Iraq.

Ismaeel, H.M. (2013). Identification of genomic markers by RAPD–PCR Primers in Iraq Breast Cancer patients. Iraq J Sci.;54:97-104.

Odero, M.D., Soto, J.L., Matutes, E., Martin-Subero, J.I., Zudaire, I., Rao, P.H., Cigudosa, J.C., Ardanaz, M.T., Chaganti, R.S., Perucho, M. and Calasanz, M.J. (2001). Comparative genomic hybridization and amplotyping by arbitrarily primed PCR in stage A B-CLL. Cancer Genet. Cytogenetic, 130: 8–13.

Papadopoulos, S., Benter, T., Anastassiou, G., Pape, M., Gerhard, S., Bornfeld, N., and Dörken, B. (2002). Assessment of genomic instability in breast cancer and uveal melanoma by random amplified polymorphic DNA analysis. International journal of cancer, 99(2), 193-200.

Saleh, N., Ibrahim, A., Archoukieh, E., Makkiya, A., Al-Obaidi, M., & Alobydi, H. (2010). Identification of genomic markers by RAPD-PCR primer in leukemia patients. Biotech, 9(2), 170-175.

Sánchez-Aguilera, A., and Méndez-Ferrer, S. (2017). The hematopoietic stem-cell niche in health and leukemia. Cellular and molecular life sciences : CMLS, 74(4), 579–590.

SAS. (2018). Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.

Scarpa, A., Moore, P.S., Rigaud, G. and Menestrina, F. (2001). Genetic alterations in primary mediastina B-cell lymphoma: an update. Leukemia and Lymphoma, 41: 47–53.

Xian, Z.H., W.M. Cong, S.H. Zhang and H.C. Wu, (2005). Genetic alteration of hepatocellular carcinoma by random amplified olymorphic DNA analysis and cloning sequencing of tumor differential DNA fragment. World J. Gastroenterol., 11: 4102-4107

Yass, N. S., and Sabah, M. A. (2012). Detection of genomic instability in oral squamous cell carcinoma using random amplified polymorphic DNA based on polymerase chain reaction method (RAPD-PCR). J. Bagh. College Dentistry. 24(2), 81.

Zhang, S.H.; Cong, W.M.; Xian, Z.H.; Dong, H. and Wu, M.C. (2004). Genomic instability in hepatocelluar carcinoma revealed by using random amplified polymorphic DNA method. J. Cancer Res. Clin. Oncol., 130 (12): 757-761.