Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2022 19(3): 1504-1511. **OPEN ACCESS**

Analysis of KRAS and NRAS in Patients with Leukemia in Saudi Arabia

Malik A. Altayar and Osama M. Al-Amer

Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, University of Tabuk, Tabuk, **Saudi Arabia**

***Correspondence:** maltayar@ut.edu.sa **Received: 29-07-2022, Revised: 19-08-2022, Accepted:24-08-2022 e-Published: 27-08-2022**

RAS genes encode highly similar proteins (H-RAS, N-RAS, and K-RAS) of 21-kDa monomers that comprise the largest group of GTPase enzymes. The role of these encoded proteins, particularly the wild type, is as on-and-off molecular switches among active GTP-bound and inactive GDP-bound states. In their active state, RAS proteins turn on genes involved in cell growth and survival. Mutations in RAS coding genes impair GTP hydrolysis activity, leading to the production of persistently active and GTP-bound RAS proteins. Three main missense substitution point mutations at codons 12, 13, and 61 account for the majority of RAS oncogenic transformational status. RAS mutations are associated with approximately 33% of human malignancies. In this study, we aimed to analyze K-RAS and N-RAS oncogene mutations in Saudi leukemia patients in the Tabuk region. A total of 62 samples of peripheral blood from leukemia malignancy patients and 100 samples from healthy controls were enrolled in this study. PCR was performed and sequenced for exons 2 and 3 to detect the three canonical mutations at codons 12, 13, and 61 in the K-RAS and N-RAS oncogenes. For the K-RAS oncogene, we did not find any mutation in either exons 2 or 3 in any leukemia patient or in the healthy controls. For the N-RAS oncogene, we did not find any mutation in exon 2. However, 6 mutations (9.7%) were found in exon 3 for N-RAS samples of leukemia patients compared with the healthy controls and the reference gene in the database. The transition mutation changed the codon GGT to codon CGT, which changed the amino acid Gly to Arg.In our study, we did not find any mutation in all known codons (12, 13 and 61) in leukemia patients. However, a mutation was found in codon number 48, and this could be a novel important mutation in leukemia patients.

Keywords: KRAS, NRAS, Leukemia

INTRODUCTION

Different proteins participate in the action of signaling networks that affect various aspects in different tissues of the human body, one of which is the regulation of cell fate. These homologous proteins, namely, HRas, NRas, KRas4a, and KRas4b (21-kDa proteins), are believed to be responsible for the latter role, encoded by RAS genes. The synthesis of the aforementioned proteins is encoded by the RAS genes, which have been shown to frequently host dominant somatic mutations in a number of human cancers (Schubbert et al.2007 and Malumbres, and Barbacid, 2003) . Atypical Ras signaling results from biochemical interactions between different molecules, such as PTPN11 and CKIT, and RAS genes, namely, N-RAS and K-RAS, which frequently contain mutations. This phenomenon is believed to be a significant therapeutic target in these cancers. Mutations in RAS are linked to cell proliferation and increased cell survival. Myeloid malignancies have been shown to be associated with various mutations in the N-RAS, K-RAS, and NF1 tumor suppressor genes, which are responsible for encoding neurofibromin. Increasing evidence also implicates these genes as “drivers” in lymphoid cancers with “high-risk” clinical features. Approximately 25% of human cancers that are believed to be associated with K-RAS or N-RAS mutations lack mechanism-based treatments (Dunbar et al.2008, Nakao, M., et al. 1996, Nakata et al. 1995, Tartaglia et al.2003) .

Ras proteins play a regulatory role in the fate of cells, by which an execution of an on/off switch between inactive and active guanosine diphosphate (GDP)-bound and guanosine triphosphate (GTP)-bound conditions occurs (Vetter, I.R. and A. Wittinghofer.2001) Following the formation of complexes on ligand binding consisting of molecules, such as Shc and Grb2, and growth factor receptors, Ras guanine nucleotide exchange factors (GEFs) are activated. GEFs regulate the dissociation and passive rebinding of guanine from Ras. The concentration of free GTP is significantly greater than the concentration of GDP in cells, which is suggested to lead to increased levels of GTP in Ras that are also involved in the action of GEF, by which nucleotide exchange is induced (Braun, and Shannon,2008). Examples of GEFs that participate in the activation of Ras are RasGRFS1, RasGRFS2, SOS1

and SOS2 (Schubbert, et al.2003) Mutations in RAS genes, particularly those located at codons 12, 13, and 61, have been reported to be highly associated with human malignancies. The reduced hydrolysis of GTP increases the tendency of these molecular variations to be more associated with the GTP-bound conformation. Regarding drug studies, it has been reported to be a significantly challenging task to discover a drug to address the oncogenic switch of Ras and GAP (Schubbert, et al.2003).

In hematologic cancers, N-RAS and K-RAS are both mutated at significant frequencies, with N-RAS mutations predominating. Mutations of N-RAS and K-RAS at codons 12, 13, or 61 have been illustrated extensively in the literature to be manifested in approximately 10% and an additional 5% among cases of adult acute myeloid leukemia (AML), respectively Lauchle, J.O., et al.2009, Bacher, U., et al. 2006). Likewise, a comparable percentage of N-RAS/K-RAS mutations with pediatric patients has been reported (Bowen, D.T., et al.2005, Vogelstein, B., et al. 1990). With regard to treatments, it has been reported that AML patients who carry a RAS mutation showed an improved response to therapy that applied after remission, which involves an elevated concentration of cytarabine. However, N-RAS and K-RAS mutations alone may not be considered independent prognostic factors in patients enrolled in plans for leukemia treatment (Berman et al. 2011). By utilizing the capability of high-throughput sequencing on samples from AML and chronic myelomonocytic leukemia (CMML) patients, a number of mutations in the N-RAS and K-RAS genes were revealed, which are suggested to be novel. Consequently, substitutions in amino acids 14, 60, 74, and 146 occurred in approximately 5% of cases. As a result, approximately 25% of AML patients harbor somatic mutations in RAS (N-RAS and K-RAS) genes (Neubauer, et al.2008 Tyner et al. 2009.)

RAS mutations have been found to be carried by almost 11% of lymphoid leukemia patients, particularly T-cell acute lymphoblastic leukemia (Flex et al.2008,and Neri et al.1998) found that 18% of (ALL) patients carried N-RAS mutations, particularly at codon 12 or 13 (Neri et al.1998) The frequency of RAS mutations in both Band T-lineage ALL was estimated to be approximately 15% (predominantly N-RAS mutations), as reported in the pediatric literature (Zhang et al.2011 and Perentesis, J.P., et al.2004). Regarding multiple myeloma (MM), mutated RAS genes are highly associated with the incidence of MM cases [Chng, W.J., et al.2009]. It was reported that the frequency of associated mutations in N-RAS and K-RAS was 30%-40% (Liu et al.1996, Corradini, P., et al.1993 and Rasmussen et al.2005) while a recent study showed that 23% of patients harbor RAS mutations at codons 12, 13, or 61 [20]. Most of these mutations are in the N-RAS gene, while K-RAS carries fewer mutations, mostly in codon 61 of the former and codons 12 and 13 of the latter. In addition, RAS mutations could be involved in MM survival. Moreover, similar to AML, the detection of RAS gene mutations occurs occasionally at relapse rather than at the diagnosis stage. These data primarily suggest that mutations in N-RAS/K-RAS could participate in MM progression instead of initiation (Chng et al. 2004 ,Liu et al.1996, Corradini et al.1993 and Rasmussen et al.2005)

To date, there are no publications demonstrating the prevalence of RAS mutations in hematologic malignancies in the Saudi population, especially in the northern region (Tabuk). In this study, we aimed to analyze RAS oncogene mutations in human leukemia malignancies in the Tabuk population.

MATERIALS AND METHODS

Study population

In this study, 62 samples were collected from clinically confined leukemia cases of Saudi patients in the western north region of Saudi Arabia and 100 healthy controls without any types of cancer. Peripheral blood samples were collected in EDTA tubes. This study was approved by the Research Ethics committees at Tabuk University.

Genotyping and sequencing

DNA was obtained from peripheral blood using a QIAamp DNA Blood Kit as described by the manufacturer's instructions (Qiagen, Valencia, CA, USA). The extracted DNA concentration and quality were determined by the ratios of A260/A280 and A260/A230 using a NanoDrop™ (Thermo Scientific, USA). PCR was conducted to amplify sequences of exons 2 and 3 for the K-RAS oncogene and amplify sequences of exons 2 and 3 for the N-RAS oncogene using the primers in Table 1. Then, PCR products were extracted and purified using the QIAquick Gel Extraction kit (QIAGEN Ltd, UK) according to the manufacturer's instructions. The purified PCR product (50 ng/µl) was sequenced using an ABI 3730 DNA capillary sequencer (Applied Biosystems) in both directions. Sequencing data were analyzed using FinchTV software.

Statistical analysis

Data analysis was performed using SPSS version 16.0 software (SPSS, Chicago, USA). The chi-square test was performed to assess the mutation status. The data were considered to be significant if the p value was less than 0.05.

RESULTS

Patient characteristics

Sixty-two samples from patients diagnosed with leukemia and one hundred healthy controls were enrolled in this study. The age range of the patients was between 28 and 75 years, and males and females were included. Only AML (50%) and CML (50%) types of leukemia were included in this study. We found mutations only in exon 3 of the N-RAS oncogene. Mutations were found in 3 samples of AML patients and in 3 samples of CML

patients, with a total of 6 (9.7%) out of 62 leukemia patients (Table 2).

Prevalence of K-RAS and N-RAS gene mutations

In this study, we analyzed exons 2 and 3 for the K-RAS oncogene and N-RAS oncogene, as they included codons 12, 13 and 61. Figure 1 shows the representative sequences of DNA and protein of exons 2 and 3 for the K-RAS oncogene. Figure 2 shows the representative sequences of DNA and protein of exons 2 and 3 for the N-RAS oncogene.

For the K-RAS oncogene, we did not find any mutation in either exons 2 or 3 in any leukemia patient or in the healthy controls. Figure 3 shows the wild-type sequencing of exons 2 and 3 for the K-RAS oncogene. For the N-RAS oncogene, we did not find any mutation in exon 2. However, 6 mutations have been found in exon 3 for N-RAS samples of lymphoma patients compared with the healthy controls and the reference gene in the database. Figure 4 presents the wild-type sequencing of exons 2 and 3 of N-RAS.

Table 3 summarizes the mutations found in exon 3 of N-RAS. The observed mutations were G-C transitions (6/62, 9.7%). The transition mutation changed the codon **G**GT to codon **C**GT, which changed the amino acid Gly to Arg. This mutation was found in codon number 48. However, the results demonstrated that there was no significant correlation found between the overall N-RAS mutation between leukemia samples and healthy controls. In addition, the data showed that there was no mutation in known codons 12, 13 and 61 in either K-RAS or N-RAS in Saudi leukemia patients. Figure 5 shows the mutation found in exon 3 of N-RAS in leukemia samples compared with the wild-type sequence.

Table 1: Primers used for K-RAS and N-RAS exons 2 and 3.

	Forward primer	Reverse primer	Size
KRAS exon 2	GTGTGACATGTTCTAATATAGTCA	GAATGGTCCTGCACCAGTAA	540 pb
KRAS exon 3	TCAAGTCCTTTGCCCATTTT	TGCATGGCATTAGCAAAGAC	594 pb
NRAS exon 2	GAACCAAATGGAAGGTCACA	TGGGTAAAGATGATCCGACA	720 pb
NRAS exon 3	GGTGAAACCTGTTTGTTGGA	AACCTAAAACCAACTCTTCCCA	720 pb

Table 2: Frequencies of exons mutations in leukemia patients

Characteristics	% (n: 62)	P value	K-RAS mutation (%)	P value	N-RAS mutation (%)	P value
Gender Male Female	50% (31/62) 50% (31/62)	1.00	0% (0/31) 0% (0/31)	1.00	9.7% (3/31) 9.7% (3/31)	1.00
Age <65 ≥65	64.5% (40/62) 35.5% (22/62)	0.002	0% (0/40) 0% (0/22)	1.00	10% (4/40) 9.1% (2/22)	1.00
Types of leukemia AML CML	50% (31/62) 50% (31/62)	1.00	0% (0/31) 0% (0/31)	1.00	9.7% (3/31) 9.7% (3/31)	1.00

Table 3: Changes found in exon 3 of N-RAS

Exon	Nucleotide change	Codon changed	No. of cogon	Amino acid changed	Frequency	Percentage
3	G>C	**G**GT > **C**GT	48	Gly48Arg	6/62	9.7%

Figure 1: Sequencing and position of exons 2 and 3 of the K-RAS oncogene.

(A) Exon 2 (Forward):

GT TCT TGC TGG TGT GAA ATG ACT GAG TAC AAA CTG GTG GTG GTT GGA GCA GGT GGT GTT
GGG AAA AGC GCA CTG ACA ATC CAG CTA ATC CAG AAC CAC TTT GTA GAT GAA TAT GAT CCC
ACC ATA GAG

(B) Exon 3 (Forward):

GAT TCT TAC AGA AAA CAA GTG GTT ATA GAT GGT GAA ACC TGT TTG TTG GAC ATA CTG GAT
ACA GCT GGA CAA GAA GAG TAC AGT GCC ATG AGA GAC CAA TAC ATG AGG ACA GGC GAA GGC
TTC CTC TGT GTA TTT GCC ATC AAT AAT AGC AAG TCA TTT GCG GAT ATT AAC CTC TAC AG

(C) Exon 3 (Reverse):

CT GTA GAG GTT AAT ATC CGC AAA TGA CTT GCT ATT ATT GAT GGC AAA TAC ACA GAG GAA
GCC TTC GCC TGT CCT CAT GTA TTG GTC TCT CAT GGC ACT GTA CTC TTC TTG TCC AGC
TGT ATC CAG TAT GTC CAA CAA ACA GGT TTC ACC ATC TAT AAC CAC TTG TTT TCT GTA
AGA ATC

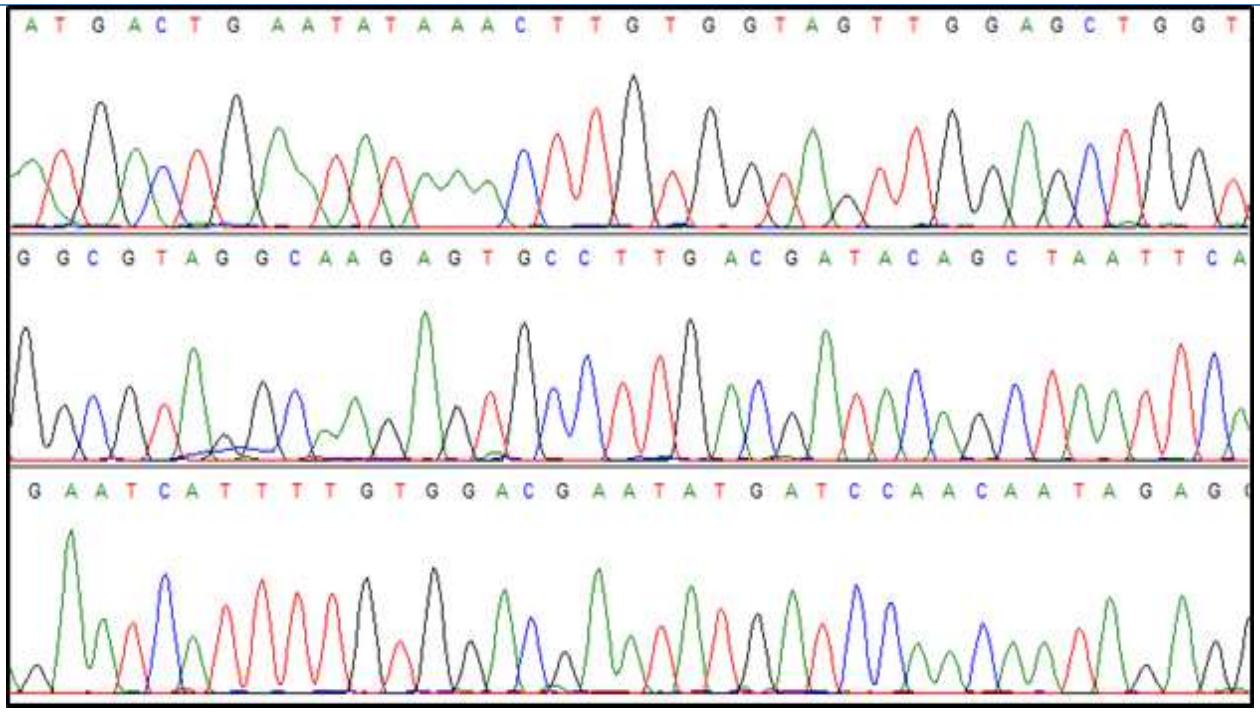
(D) Protein:

MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAGQEEYSAMRDQYMRTGEGF
LCVFAINNSKSFADINLYREQIKRVKDSDDVPMVLVGNKCDLPTRTVDTKQAHELAKSYGIPFIETSAKTRQGVEDAF
YTLVREIRQYRMKKLNSSDDGTQGCMGLPCVVM

(E) Positions of each exon:

Exon 1 5001 – 5237
Exon 2 5718 – 5845
Exon 3 7917 – 8095

Figure 2: Sequencing and position of exons 2 and 3 of the N-RAS oncogene.



(B)

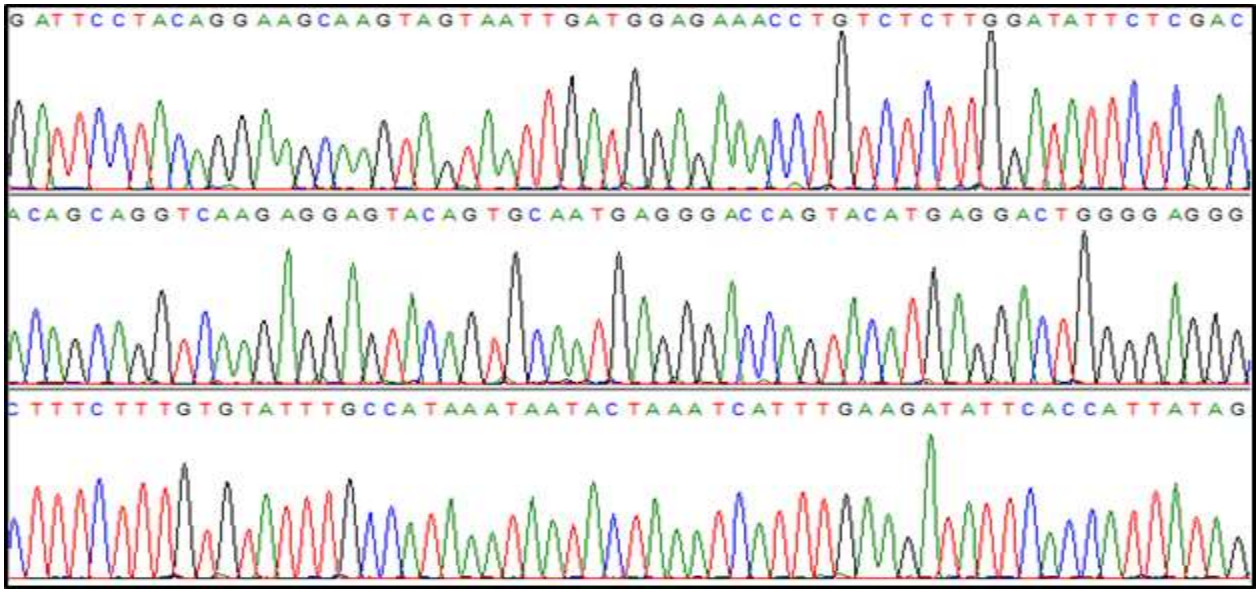


Figure 3: The type sequence of exon 2 and exon 3 of the K-RAS oncogene.

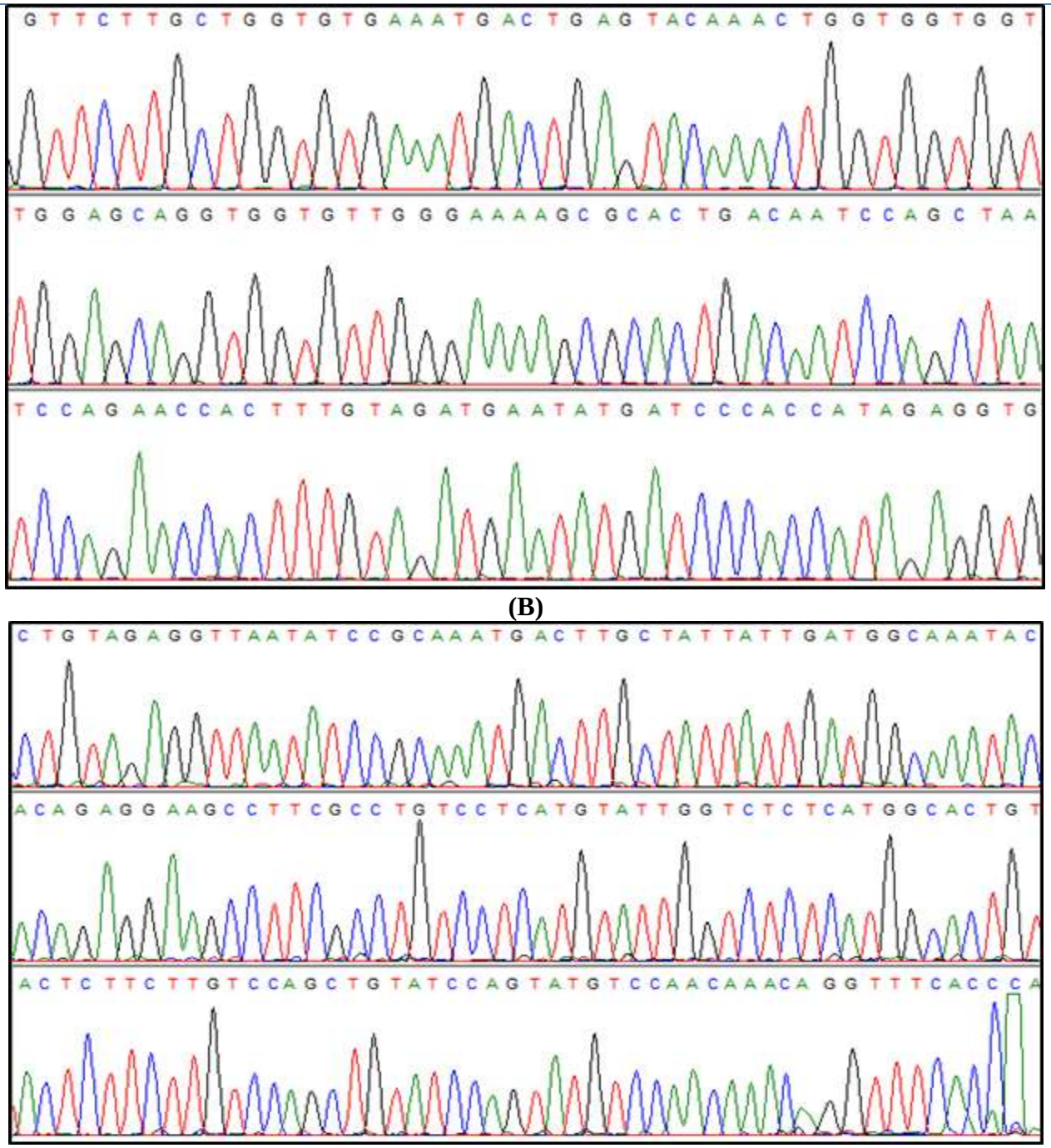


Figure 4: The type sequence of exons 2 and exon 3 of the N-RAS oncogene.

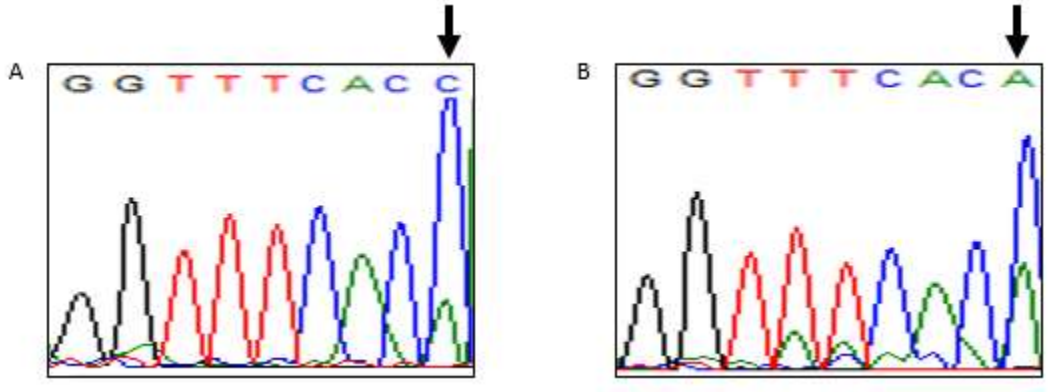


Figure 5: Transition mutation detected in exon 3 of the N-RAS oncogene.

DISCUSSION

Many studies have indicated the role of the RAS oncogene in different types of tumors. Mutations in K-RAS and N-RAS were reported in colorectal cancer patients. Mutations are mainly found in codons 12, 13 and 61 (Naser, W.M., et al. 2014, Baskin, Y., et al. 20014, Zocche, D.M., et al. 2015, Taniguchi, H., et al. 2015). Mutations of K-RAS and N-RAS were also reported in other solid tumors, including hepatocellular carcinoma, lung cancer, ductal adenocarcinoma, endocrine tumor, adenocarcinoma, malignant melanoma, anaplastic carcinoma, follicular carcinoma, and lung cell carcinoma et al. 2012). RAS mutations are not only found in solid tumors. Additionally, many reported RAS oncogenes have a role in hematologic malignancies. K-RAS and N-RAS mutations have been reported in different hematologic malignancies, including JMML, CMML, AML, MDS, ALL, MM and others (Ward et al. 2012 and Steinbrunn, et al.2011).

This study aimed to determine the prevalence of oncogenic RAS mutations in patients with lymphoid leukemia in the Saudi population in the Tabuk region. This is the first study of exons 2 and 3 of K-RAS and N-RAS in leukemia patients in the Tabuk region. Exons 2 and 3 of 62 patients diagnosed with leukemia and 100 healthy controls were enrolled in this study. We did not detect any mutations in any of the leukemia and healthy control samples in codons 12, 13 and 61. Six mutations found in exon 3 of the N-RAS oncogene were related to leukemia samples. These mutations were found in codon number 48, in which the **G**GT codon was changed to the **C**GT codon, which ultimately changed the Gly amino acid to an Arg amino acid in the protein sequence.

CONCLUSION

The current study found that mutations in codons 12, 13 and/or 61 are not associated with the incidence of leukemia malignances in Saudi Arabia. Mutation in codon 48 of the N-RAS oncogene could be related to the incidence of leukemia in Saudi Arabia. However, in the future, more samples should be included in the studies.

CONFLICT OF INTEREST

The author declares no conflict of interest.

ACKNOWLEDGEMENT

The authors would like to thank the University of Tabuk.

AUTHOR CONTRIBUTIONS

All authors designed and participated in the experiments. All authors participated in analysing the data, writing, editing and reviewing the manuscript. All authors read and approved the final version of the manuscript.

REFERENCES

Bacher, U., et al. *Implications of NRAS mutations in AML: a study of 2502 patients.* Blood, 2006. 107(10): p. 3847-53.

Baskin, Y., et al. *KRAS and BRAF mutation frequencies in a series of Turkish colorectal cancer patients.* Translational Cancer Research, 2014. 3(2): p. 160-166.

Berman, J.N., et al. *Prevalence and clinical implications of NRAS mutations in childhood AML: a report from the Children's Oncology Group.* Leukemia, 2011. 25(6): p. 1039-42.

Bowen, D.T., et al. *RAS mutation in acute myeloid leukemia is associated with distinct cytogenetic subgroups but does not influence outcome in patients younger than 60 years.* Blood, 2005. 106(6): p. 2113-9.

Braun, B.S. and K. Shannon, *Targeting Ras in myeloid*

leukemias. Clin Cancer Res, 2008. 14(8): p. 2249-52.

Chng, W.J., et al. *Clinical and biological significance of RAS mutations in multiple myeloma.* Leukemia, 2008. 22(12): p. 2280-4.

Corradini, P., et al. *Mutational activation of N- and K-ras oncogenes in plasma cell dyscrasias.* Blood, 1993. 81(10): p. 2708-13.

Dunbar, A.J., et al. *250K single nucleotide polymorphism array karyotyping identifies acquired uniparental disomy and homozygous mutations, including novel missense substitutions of c-Cbl, in myeloid malignancies.* Cancer Res, 2008. 68(24): p. 10349-57.

Fernandez-Medarde, A. and E. Santos, *Ras in cancer and developmental diseases.* Genes Cancer, 2011. 2(3): p. 344-58.

Flex, E., et al. *Somatically acquired JAK1 mutations in adult acute lymphoblastic leukemia.* J Exp Med, 2008. 205(4): p. 751-8.

Lauchle, J.O., et al. *Response and resistance to MEK inhibition in leukaemias initiated by hyperactive Ras.* Nature, 2009. 461(7262): p. 411-4.

Liu, P., et al. *Activating mutations of N- and K-ras in multiple myeloma show different clinical associations: analysis of the Eastern Cooperative Oncology Group Phase III Trial.* Blood, 1996. 88(7): p. 2699-706.

Malumbres, M. and M. Barbacid, *RAS oncogenes: the first 30 years.* Nat Rev Cancer, 2003. 3(6): p. 459-65.

Nakao, M., et al. *Internal tandem duplication of the flt3 gene found in acute myeloid leukemia.* Leukemia, 1996. 10(12): p. 1911-8.

Nakata, Y., et al. *c-kit point mutation of extracellular domain in patients with myeloproliferative disorders.* Br J Haematol, 1995. 91(3): p. 661-3.

Naser, W.M., et al. *Novel KRAS gene mutations in sporadic colorectal cancer.* PLoS One, 2014. 9(11): p. e113350.

Neri, A., et al. *Analysis of RAS oncogene mutations in human lymphoid malignancies.* Proc Natl Acad Sci U S A, 1988. 85(23): p. 9268-72.

Neubauer, A., et al. *Patients with acute myeloid leukemia and RAS mutations benefit most from postremission high-dose cytarabine: a Cancer and Leukemia Group B study.* J Clin Oncol, 2008. 26(28): p. 4603-9.

Perentesis, J.P., et al. *RAS oncogene mutations and outcome of therapy for childhood acute lymphoblastic leukemia.* Leukemia, 2004. 18(4): p. 685-92.

Prior, I.A., P.D. Lewis, and C. Mattos, *A comprehensive survey of Ras mutations in cancer.* Cancer Res, 2012. 72(10): p. 2457-67.

Rasmussen, T., et al. *Possible roles for activating RAS mutations in the MGUS to MM transition and in the intramedullary to extramedullary transition in some plasma cell tumors.* Blood, 2005. 105(1): p. 317-23.

Schubbert, S., K. Shannon, and G. Bollag, *Hyperactive Ras in developmental disorders and cancer.* Nat Rev Cancer, 2007. 7(4): p. 295-308.

Steinbrunn, T., et al. *Mutated RAS and constitutively activated Akt delineate distinct oncogenic pathways, which independently contribute to multiple myeloma cell survival.* Blood, 2011. 117(6): p. 1998-2004.

Taniguchi, H., et al. *Japanese Society of Medical Oncology Clinical Guidelines: RAS (KRAS/NRAS) mutation testing in colorectal cancer patients.* Cancer Sci, 2015. 106(3): p. 324-7.

Tartaglia, M., et al. *Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia.* Nat Genet, 2003. 34(2): p. 148-50.

Tyner, J.W., et al. *High-throughput sequencing screen reveals novel, transforming RAS mutations in myeloid leukemia patients.* Blood, 2009. 113(8): p. 1749-55.

Vetter, I.R. and A. Wittinghofer, *The guanine nucleotide-binding switch in three dimensions.* Science, 2001. 294(5545): p. 1299-304.

Vogelstein, B., et al. *RAS gene mutations in childhood acute myeloid leukemia: a Pediatric Oncology Group study.* Genes Chromosomes Cancer, 1990. 2(2): p. 159-62.

Ward, A.F., B.S. Braun, and K.M. Shannon, *Targeting oncogenic Ras signaling in hematologic malignancies.* Blood, 2012. 120(17): p. 3397-406.

Zhang, J., et al. *The genetic basis of early T-cell precursor acute lymphoblastic leukaemia.* Nature, 2012. 481(7380): p. 157-63.

Zocche, D.M., et al. *Global impact of KRAS mutation patterns in FOLFOX treated metastatic colorectal cancer.* Front Genet, 2015. 6: p. 116.