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VALIDATION OF RS 9971 SINGLE NUCLEOTIDE POLYMORPHISM (SNP) IN BLOOD CANCER PATIENTS OF HYDERABAD – INDIA

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1. ABSTRACT

Cancer, the uncontrolled growth of cells leading to highly abnormal cell metabolism, is one of the major health hazards especially being the cause of death in most of the countries. Aim of the present work is to study rs 9971 Single Nucleotide Polymorphism (SNP) in the MCL1 gene, involved in Myeloid Leukemia, and their comparative presence in the diseased and control samples. The mutation causes the change in the single nucleotide from G to T leading to the change in the protein code of MCL 1 gene. Total 30 samples were collected for investigation in which 20 were diseased and 10 were control. DNA was extracted from all the samples and the purification was performed. Standard gene sequence of MCL 1 was retrieved from NCBI database and the SNP was identified in it. Specific primers were designed for the segment containing SNP. PCR was performed and the amplified products were sent for sequencing. The sequences were analyzed for the presence of SNP. SNP rs9971 was observed to be present in only three out of twenty diseased samples. This study can be laid as the basis for developing a diagnostic tool for the identification of myeloid leukemia.

KEYWORDS: Myeloid Leukemia, MCL 1 gene, Single Nucleotide Polymorphism, rs 9177.

2. INTRODUCTION

In normal homeostasis, in a multicellular organism, formation of new cells and removal of old cells occur in a tightly controlled manner. Regulation of these two processes is maintained by a number of checkpoints and also by many gene products. These checkpoints and gene products act as signals which control the cell replication process. Whenever these checkpoints or genes are deregulated, leads to a stage of uncontrolled growth of cells.[1]

Cancer is a stage in which a cascade of cellular events leads to alteration of normal functioning of cell. This alteration is caused by modifying the normal molecular events. These altered cells do not obey the traditional regulation checkpoints.[2] These modifications in cellular system cause them to multiply in an irregular and uncontrolled manner. Another property gained by cancerous cells is the ability to invade other tissues. Few of the many properties possessed by these cancerous cells are cell structure modification, loss of contact inhibition and also there are changes in types and amounts of gene expression. A variety of causes are responsible for these alterations. To name a few mutations, radiation, physical agents and viral infections and heredity. A cell becomes cancerous when the changes occur in protein encoding genes, these proteins essentially control the duplication of cell. When a cell undergoes changes in its genetic material (DNA), the same changes are inherited by all the progeny cells. When a variety of these changes accumulate, cancerous cells gain the ability of dividing much more rapidly. These rapidly proliferating cells forms a mass, which is called as tumour. These cells when localised to their origin site are called as benign. These tumour cells become invasive which have the ability of metastasis. The next stage after malignant tumour formation is metastasis. During the metastasis stage, the malignant cells spread from their origin site to a distant site. This spread occurs mainly via lymphatic and blood circulation system. These metastasised cells are invasive in nature.[3]

2.1 BLOOD CANCER

Blood cancer is a collection of types of cancer which affects different types of cells in the blood. Mainly bone marrow is the centre for start of blood cancer as all the blood cells originate from this part. The main types of blood cells produced from bone marrow are red blood cells, white blood cells and platelets. In case of any type of blood cancer, one of the blood cell is altered leading to a deregulated growth pattern. This result in interruption of normal blood cells development and also alters the functions carried out by blood and its components. Basically, leukemia is categorised into two: acute, characterised by aggressive disease progression and faster fatal result and chronic, slow progression of cancer and more difficult to treat. There are mainly three types of blood cancer which are categorised based on the type of blood cell involved. First is Leukemia, this form basically involves excess proliferation of white blood cells. These excess numbers of altered WBCs do not function properly and this also results in impaired production of RBCs and also platelets. Next is Lymphoma, which is mainly characterised by overproduction of abnormal immune cells called as lymphocytes (cells involved in immune system, a type of WBCs). As a result,

immunity of a person is compromised in this type of blood cancer. Lymphoma affects the lymphatic system. Myeloma is another kind of blood cancer in which antibody producing cells called as plasma cells are affected. This condition also leads to compromised immunity.[4-7]

Chronic Myelogenous Leukemia (Chronic Myeloid Leukemia), a form of blood cancer originates from the bone marrow from newly proliferating blood cells.[8] CML is linked to a genetic alteration, basically a chromosomal translocation as its causative factor. This translocation results in the fusion of a gene on chromosome 9 called as Abelson Murine Leukemia (ABL) gene with another gene on chromosome 22 called as Breakpoint Cluster Region (BCR). This translocation results in the formation of chromosome 22 but decreased in length called as Philadelphia Chromosome.[9] As a result of this gene fusion, an oncoprotein is expressed called as BCR-ABL. This oncoprotein is a tyrosine kinase which is constitutive in its expression. As it is a tyrosine kinase and also constitutively expressed, it enhances proliferation of the cell by downstream signaling through RAS, RAF, JUN kinase, MYC, and STAT. This leads to cell proliferation cycle which is independent of cellular signals like cytokines. Another supporting factor for the deregulated proliferation is ineffective or abnormal apoptotic signal.[10-16]

Single nucleotide Polymorphisms (SNPs) are considered as one of the main causes for genetic alterations, which is one of the important cause of cancer. A single base substitution (mutation) can be considered as an SNP when the same substitution is observed at the specific site in a population greater than 1%. A majority of SNPs do not cause any sort of physical alteration in the gene expression as they are inherited. As these are inherited, they can also act as genetic markers. Therefore stress has been laid on to identify those single point mutations which have an effect on gene expression and in turn on its product.[17]

SNPs have a prominent role in cancer research. These can be associated genetically with cancer mainly two ways, first in susceptibility search and also in prediction of outcome. In susceptibility search, an individual is examined on the basis of SNPs detected and interaction of these SNPs with environmental factors which could probably result in cancer. This approach mainly emphasize on gene (SNP) - environment interaction which results in a stress to the individual and also intensifies the physical alteration that can be caused by that particular SNP. Not all the SNPs are cancer causing, some of them can be protective also. In

prediction of outcome, these SNPs could also help in prediction of cancerous outcome (metastatic or aggressive) .[18,19]

SNPs are involved in pharmacogenomics, a field which investigates the differences in response to a drug by individuals based on their genetic make up. Pharmacogenomics is applies basically in two approaches. First is to identify the individual's response to a particular drug based on their genes. Second is to estimate the efficiency of the drug in consideration on a particular individual which is also inturn decided by their genes. Based on these two approaches, the type of drug to be used for an individual is determined.[20] There have been a number of reports suggesting a positive relation between SNPs and chronic myelogenous leukemia (CML). One of the study was conducted by Claudia Bănescu et al[21] reported a positive relation between Xeroderma Pigmentosum Complementation group D (XPD) repair genes Lys751Gln polymorphism and CML risk.

MCL 1 (Myeloid Cell Leukemia 1) gene codes for a protein called as Induced myeloid leukemia cell differentiation protein Mcl-1. This well characterised gene is one of the several members from BCL 2 family.[22] BCL-2 family, whose main function is to regulate cell proliferation cascade, are main mediators of cellular responses against DNA Damage.[23, 24] This family consists of many members, which can be broadly categorised into two groups based on their function. First is anti apoptotic, which inhibits the programmed cell death includes MCL-1, BXL-X, BXL-W and BCL-B. Second is pro-apoptotic which induce the apoptosis program which have two sub groups: pro apoptotic activators and pro apoptotic effectors. Pro apoptotic activators includes BID, BIM, PUMA, NOXA and BAD. BAX, BAK and BOK comprises of the group pro apoptotic effectors. There is always a dynamic regulation and balance between expression and interaction of these pro apoptotic members and anti apoptotic members which directs the cell whether to continue for apoptosis or not. During apoptosis, interaction between pro apoptotic activators and effectors leads to cell death. But in normal cell, this interaction is inhibited by BCL-2 as it directly binds to pro apoptotic activators resulting in loss of interaction between activators and effectors.[25-28]

Apart from being involved in apoptosis regulation, MCL 1 has several other functions which includes its role in development and differentiation of B and T lymphocytes, neural development[29-33] and also it decelerates the cell proliferation pathway by its interaction through CDK1, CHK 1 and PCNA.[34-36]

The main role of MCL 1 gene product was determined to be anti-apoptotic in nature as was found in many studies using different cell lines of leukemia.[37, 40-43] Similar results were obtained by Kozopas KM et al[22] when they induced differentiation of leukemia ML-1 cells with 12-O-tetra decanoyl phorbol 13-acetate (TPA). Results of all these studies stress on the factor that MCL-1 protein act for enhancing survival of cell and is an anti apoptotic molecule. Karl J. Aichberger et al[37] showed that MCL 1 expression is enhanced by BCR-ABL expression in turn leading to activation cell proliferation promoting pathways activation like RAS/RAF/MAP kinase pathways. They also proved through their studies that MCL 1 has a survival enhancing activity because the K562 cell viability levels decreased when MCL 1 expression was inhibited by using MCL 1 siRNA and specific antisense nucleotides. MCL 1 gene expression is proved to be cell specific and differentiation stage specific. MCL 1 expression was observed in immature cells of erythroid and lymphoid lineages but was not observed in mature stages.[38, 39, 43] As a conclusion of all these studied conducted at various levels, MCL 1 can be considered as a candidate gene for further research in myeloid leukemia.

The single nucleotide polymorphism considered in this study is rs7791 in MCL 1 gene. This polymorphism basically results in a change of one nucleotide at position of 448th from the original Guanine to thymine.[44] This modification of nucleotides resulting change of amino acid residue for that particular codon from Asp to Tyr. This sort of SNP was observed in many cases of myeloid leukemia making it a potential site for protein docking which can be used for drug designing and also a potential biomarker for identification of myeloid leukemia.

3. MATERIALS AND METHODS

3.1 SAMPLE COLLECTION

Blood samples 30 which include 20 Myeloid Leukemia and 10 normal control individuals were collected from a Diagnostic centre located at LB Nagar, Hyderabad, India.

3.2 GENOMIC DNA EXTRACTION

Bunce method was used for Genomic DNA extraction. In this method, 1 ml of anticoagulated blood was taken and 3 ml of Solution 1 (100 ml; 1M Tris HCl, Sucrose-10gms, MgCl2-47 mg), pH-8 was added. The the vial was slowly mixed till 5 mins then incubated at 37 degrees for 5 mins. Spin was carried out at 6000 rpm for 5mins. To the pellet, 2 volumes of solution B (100 ml; 1M Tris HCl, 0.5M EDTA and NaCl-0.876gms) pH-8 was added. The pellet was resuspended in solution B. Then the sample was incubated for 30 mins at 37 degrees. Then

3M Sodium acetate was added (650µl). Then the sample was incubated at 65 degrees for 20mins. Then 650 µl chloroform was added. The sample was slowly mixed for 60 mins. Spin was done at 6000 rpm for 10 mins. To the supernatant, equal volume of isopropanol was added so that the DNA gets precipitated as white fibres. Then centrifugation was carried at 12000 rpm for 5 mins for the DNA to get pelleted at the bottom of the vial. Was the pellet with 70% Ethanol by centrifuging at 10000 rpm for 5 mins. The pellet was air dried and was dissolved in TE Buffer. For confirmation of DNA extracted, agarose gel electrophoresis was performed and purity was checked.

3.3 PRIMER DESIGNING

For primer designing, MCL 1 gene sequence was obtained from NCBI database.

MCL 1 gene sequence

Name of the Gene: MCL1

Chromosome: 1

Starting: 150574551 to c150579738

Arm: q

>gi|568815597:c150579738-150574551 Homo sapiens chromosome 1, GRCh38.p2 Primary Assembly

GCGCAACCCTCCGGAAGCTGCCGCCCCTTTCCCCTTTTATGGGAATACTTTTTTTA
AAAAAAAAGAGTTCGCTGGCGCCACCCCGTAGGACTGGCCGCCCTAAAACCGTG
ATAAAGGAGCTGCTCGCCACTTCTCACTTCCGCTTCCTTCCAGTAAGGAGTCGGG
GTCTTCCCCAGTTTTCTCAGCCAGGCGGCGGCGGCGACTGGCAATGTTTGGCCTC
AAAAGAAACGCGGTAATCGGACTCAACCTCTACTGTGGGGGGGCCGGCTTGGGG
GCCGGCAGCGGCGGCGCCACCCGCCCGGGAGGGCGACTTTTGGCTACGGAGAAG
GAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGGGAGGCCGGCGCGGTGATTGG
CGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTCACGCCAGACTCCCGGAGGGT
CGCGCGGCCGCC**G**CCCATTGGCGCCGAGGTCCCCGACGTCACCGCGACCCCCGC
GAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGGCGCCGCTTGAGGAGATGGAA
GCCCCGGCCGCTGACGCCATCATGTCGCCCGAAGAGGAGCTGGACGGGTACGAG
CCGGAGCCTCTCGGGAAGCGGCCGGCTGTCCTGCCGCTGCTGGAGTTGGTCGGG
GAATCTGGTAATAACACCAGTACGGACGGGTCACTACCCTCGACGCCGCCGCCA
GCAGAGGAGGAGGAGGACGAGTTGTACCGGCAGTCGCTGGAGATTATCTCTCGG
TACCTTCGGGAGCAGGCCACCGGCGCCAAGGACACAAAGCCAATGGGCAGGTCT

GGGGCCACCAGCAGGAAGGCGCTGGAGACCTTACGACGGGTTGGGGATGGCGTG
CAGCGCAACCACGAGACGGCCTTCCAAGGTAAGGGGGTTCATTAATCGCCAAGG
CCTCACTCCCTTTTTTCCATCTCTCCCCGGACTCACCCGCCAAGGGTGGGTTGGAA
ACCGAAACGAGTCAGTGTTGAAACGTGTCTCATCCTATTCCTGAAGCCAGAATAT
TCTGGCCATGAGTCATTGTTTCCGCCCATCTTGATTCTTTTGGAAATGGCAGCTCT
TGTTCAAAGACCGGAAAGGGTGGGATGTCAATTTCAAGTGGGGTCAACCTGAGT
TCTGTAAATCCCAGTAGCGATTTTCCCGCCGCGGGTGGGCAGGCGAATCTTGCGC
CGGTTTAGACAAAGGAGGCCGTGAGGACCTGCATGCTTTTCTTTCTCAGGCATGC
TTCGGAAACTGGACATCAAAAACGAAGACGATGTGAAATCGTTGTCTCGAGTGA
TGATCCATGTTTTCAGCGACGGCGTAACAAACTGGGGCAGGATTGTGACTCTCAT
TTCTTTTGGTGCCTTTGTGGCTAAACACTTGAAGACCATAAACCAAGAAAGCTGC
ATCGAACCATTAGCAGAAAGTATCACAGACGTTCTCGTAAGGACAAAACGGGAC
TGGCTAGTTAAACAAAGAGGCTGGGTAAGTTTGCCTTAAGGATGAAAGGGGCCT
TGGAGTGGAAGTAGAATGAAGGATTTTTTTTAGAGAGGTGGGGATATCTAAAGG
TTTTTATGACGCACGGCTGTTTGCAGGCTCTAACTAAAGGACCATTGTTTATTTGA
TGTTGATTTAAGTAGTGGATCCTTAGAGATAGTGGTATGGCGGTCTTGAATTGTA
TCAAAAATCTTGGTTTTCTCTAGGCAATTTTTTGTTCCAATTCAGTTGAATACTCT
TCAGTGGATTCAAACCATGAAAAAATAAGTCACCAGGGGAGGATAGCTGAAATA
ATTCCTAAGGCGGTGCCTGTTTTAATGGAGAAGATATGGGGTGGAGCCTGCGTTT
TAAACAAACCCAGATCTGATGCAGGATGTACTTAACTACGTTGAGAAAAACTGA
TCTGCGCAATTGAGGCGTTACTGAAATATTAGGTGGTGGAGATTTGAGAATAAG
GGTTTTCGTCTTTTACCTCATGGGAACTCTGGAAGTCCTTTTGTTAGGATAAATCC
TAATAAGACCAAGATAGTACTGTAAAATGAAGTTTAATTATCATGGGTCCCCGCT
TAAGAAACTGAAGAACTTATTTTCTTTTTTTGCCCCGGGGTGAATAATAATTGGTT
TACTATTGCTTTAGGGGGAAACCTTAGATATTTTAATTTACCTTCTCTCTGGATAG
TAGTGTTGTAAGAGAGCAGAAACCCATACTTGAAAATGTGCTTTTCTTTTTTGTTT
TCTAGGATGGGTTTGTGGAGTTCTTCCATGTAGAGGACCTAGAAGGTGGCATCAG
GAATGTGCTGCTGGCTTTTGCAGGTGTTGCTGGAGTAGGAGCTGGTTTGGCATAT
CTAATAAGATAGCCTTACTGTAAGTGCAATAGTTGACTTTTAACCAACCACCACC
ACCACCAAAACCAGTTTATGCAGTTGGACTCCAAGCTGTAACTTCCTAGAGTTGC
ACCCTAGCAACCTAGCCAGAAAAGCAAGTGGCAAGAGGATTATGGCTAACAAGA
ATAAATACATGGGAAGAGTGCTCCCCATTGATTGAAGAGTCACTGTCTGAAAGA
AGCAAAGTTCAGTTTCAGCAACAAACAAACTTTGTTTGGGAAGCTATGGAGGAG
GACTTTTAGATTTAGTGAAGATGGTAGGGTGGAAAGACTTAATTTCCTTGTTGAG

AACAGGAAAGTGGCCAGTAGCCAGGCAAGTCATAGAATTGATTACCCGCCGAAT
TCATTAATTTACTGTAGTGTTAAGAGAAGCACTAAGAATGCCAGTGACCTGTGTA
AAAGTTACAAGTAATAGAACTATGACTGTAAGCCTCAGTACTGTACAAGGGAAG
CTTTTCCTCTCTCTAATTAGCTTTCCCAGTATACTTCTTAGAAAGTCCAAGTGTTC
AGGACTTTTATACCTGTTATACTTTGGCTTGGTTTCCATGATTCTTACTTTATTAGC
CTAGTTTATCACCAATAATACTTGACGGAAGGCTCAGTAATTAGTTATGAATATG
GATATCCTCAATTCTTAAGACAGCTTGTAAATGTATTTGTAAAAATTGTATATATT
TTTACAGAAAGTCTATTTCTTTGAAACGAAGGAAGTATCGAATTTACATTAGTTT
TTTTCATACCCTTTTGAACTTTGCAACTTCCGTAATTAGGAACCTGTTTCTTACAG
CTTTTCTATGCTAAACTTTGTTCTGTTCAGTTCTAGAGTGTATACAGAACGAATTG
ATGTGTAACTGTATGCAGACTGGTTGTAGTGGAACAAATCTGATAACTATGCAGG
TTTAAATTTTCTTATCTGATTTTGGTAAGTATTCCTTAGGTTTTTCTTTGAAAACCT
GGGATTGAGAGGTTGATGAATGGAAATTCTTTCACTTCATTATATGCAAGTTTTC
AATAATTAGGTCTAAGTGGAGTTTTAAGGTTACTGATGACTTACAAATAATGGGC
TCTGATTGGGCAATACTCATTTGAGTTCCTTCCATTTGACCTAATTTAACTGGTGA
AATTTAAAGTGAATTCATGGGCTCATCTTTAAAGCTTTTACTAAAAGATTTTCAG
CTGAATGGAACTCATTAGCTGTGTGCATATAAAAAGATCACATCAGGTGGATGG
AGAGACATTTGATCCCTTGTTTGCTTAATAAATTATAAAATGATGGCTTGGAAAA
GCAGGCTAGTCTAACCATGGTGCTATTATTAGGCTTGCTTGTTACACACACAGGT
CTAAGCCTAGTATGTCAATAAAGCAAATACTTACTGTTTTGTTTCTATTAATGATT
CCCAAACCTTGTTGCAAGTTTTTGCATTGGCATCTTTGGATTTCAGTCTTGATGTT
TGTTCTATCAGACTTAACCTTTTATTTCCTGTCCTTCCTTGAAATTGCTGATTGTTC
TGCTCCCTCTACAGATATTTATATCAATTCCTACAGCTTTCCCCTGCCATCCCTGA
ACTCTTTCTAGCCCTTTTAGATTTTGGCACTGTGAAACCCCTGCTGGAAACCTGAG
TGACCCTCCCTCCCCACCAAGAGTCCACAGACCTTTCATCTTTCACGAACTTGATC
CTGTTAGCAGGTGGTAATACCATGGGTGCTGTGACACTAACAGTCATTGAGAGGT
GGGAGGAAGTCCCTTTTCCTTGGACTGGTATCTTTTCAACTATTGTTTTATCCTGT
CTTTGGGGGCAATGTGTCAAAAGTCCCCTCAGGAATTTTCAGAGGAAAGAACATT
TTATGAGGCTTTCTCTAAAGTTTCCTTTGTATAGGAGTATGCTCACTTAAATTTAC
AGAAAGAGGTGAGCTGTGTTAAACCTCAGAGTTTAAAAGCTACTGATAAACTGA
AGAAAGTGTCTATATTGGAACTAGGGTCATTTGAAAGCTTCAGTCTCGGAACATG
ACCTTTAGTCTGTGGACTCCATTTAAAAATAGGTATGAATAAGATGACTAAGAAT
GTAATGGGGAAGAACTGCCCTGCCTGCCCATCTCAGAGCCATAAGGTCATCTTTG
CTAGAGCTATTTTTACCTATGTATTTATCGTTCTTGATCATAAGCCGCTTATTTAT

ATCATGTATCTCTAAGGACCTAAAAGCACTTTATGTAGTTTTTAATTAATCTTAAG
ATCTGGTTACGGTAACTAAAAAAGCCTGTCTGCCAAATCCAGTGGAAACAAGTG
CATAGATGTGAATTGGTTTTTAGGGGCCCCACTTCCCAATTCATTAGGTATGACT
GTGGAAATACAGACAAGGATCTTAGTTGATATTTTGGGCTTGGGGCAGTGAGGG
CTTAGGACACCCCAAGTGGTTTGGGAAAGGAGGAGGGGAGTGGTGGGTTTATAG
GGGGAGGAGGAGGCAGGTGGTCTAAGTGCTGACTGGCTACGTAGTTCGGGCAAA
TCCTCCAAAAGGGAAAGGGAGGATTTGCTTAGAAGGATGGCGCTCCCAGTGACT
ACTTTTTGACTTCTGTTTGTCTTACGCTTCTCTCAGGGAAAAACATGCAGTCCTCT
AGTGTTTCATGTACATTCTGTGGGGGGTGAACACCTTGGTTCTGGTTAAACAGCT
GTACTTTTGATAGCTGTGCCAGGAAGGGTTAGGACCAACTACAAATTAATGTTGG
TTGTCAAATGTAGTGTGTTTCCCTAACTTTCTGTTTTTCCTGAGAAAAAAAAATAA
ATCTTTTATTCAAATACAGGG

Selection of SNP

rs9971

AAGCCCCCCGTCCACCCTCACGCCA[G/T]ACTCCCGGAGGGTCGCGCGGCCGCC

Chromosome:1:150579317

Gene:MCL1

Functional Consequence:intron variant,missense

Selected Region

150579250 to 150579350

>gi|568815597:c150579350-150579250 Homo sapiens chromosome 1, GRCh38.p2 Primary Assembly

GCCGGCGCAAGCCCCCCGTCCACCCTCACGCCA**G**ACTCCCGGAGGGTCGCGCGG
CCGCCGCCCATTGGCGCCGAGGTCCCCGACGTCACCGCGACCCCCGC

Specific primers were designed spanning the specific area of SNP under investigation. The primers were designed using Primer 3 software tool.

Left Primer - CGACTTTTGGCTACGGAGAA Right Primer - GGCTTCCATCTCCTCAAGC

These primers gave a predicted product size of 240bp.

3.4 POLYMERASE CHAIN REACTION AND SEQUENCING OF PRODUCTS:

The extracted DNA from all the samples were amplified using the primers designed for amplification of the particular gene MCL 1.

The resulting amplified PCR products were further sent for sequencing. Resulting sequences were analysed by a Bioinformatic tool called as CLUSTAL W. This tool aligns multiple sequences to identify the sequence matches and mis matches.

4. RESULTS

The following sequences are the PCR products of all Myeloid Leukemia and control samples.

Sequence of Myeloid Leukemia sample 1

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCC**T**CCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 2

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 3

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 4

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCC**T**CCCATTGGCGCCGAGGTCCCC

GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 5

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 6

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 7

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 8

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 9

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC

GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 10

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 11

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 12

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 13

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 14

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC

GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 15

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 16

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 17

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 18

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 19

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCC**T**CCCATTGGCGCCGAGGTCCCC

GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of Myeloid Leukemia sample 20

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of control sample 21

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of control sample 22

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of control sample 23

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of control sample 24

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC

GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of control sample 25

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of control sample 26

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of control sample 27

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of control sample 28

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of control sample 29

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC

GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequence of control sample 30

CGACTTTTGGCTACGGAGAAGGAGGCCTCGGCCCGGCGAGAGATAGGGGGAGGG
GAGGCCGGCGCGGTGATTGGCGGAAGCGCCGGCGCAAGCCCCCCGTCCACCCTC
ACGCCAGACTCCCGGAGGGTCGCGCGGCCGCCGCCCATTGGCGCCGAGGTCCCC
GACGTCACCGCGACCCCCGCGAGGCTGCTTTTCTTCGCGCCCACCCGCCGCGCGG
CGCCGCTTGAGGAGATGGAAGCC

Sequences from 1 to 20 belong to Myeloid Leukemia Patients and 21 to 30 belong to control samples. Using CLUSTAL W, a multiple sequence alignment tool, all the sequences were aligned. SNP rs 9971 was observed only in sample 1, 4 and 19.

4. DISCUSSION

Cancer, one of the leading cause of deaths in the world, results from changes at cellular levels. These changes are mainly caused by mutations at nucleotide level. These alterations results in change in type and expression levels of respective gene leading to abnormal gene products. These mutations makes the cell resistant to various cellular signals which regulate the rate of cellular proliferation. Various candidate genes and respective mutations have been identified for many cancers. Based on the identification of mutations and resulting changes in gene activity, novel drugs can be designed which particularly act on the specific mutated sites. In the present study, MCL 1 gene, a candidate gene in myeloid leukemia has been considered. Specifically single nucleotide polymorphism rs 9971 is studied in the gene which causes a single nucleotide substitution from G to T.

This gene is studied in a group of 30 people in which 20 are myeloid leukemia patients and remaining 10 are controls. After blood collection and DNA extraction, detection of SNP rs 9971 was done by specifically amplifying the MCL 1 gene and by sequence analysis. After alignment and analysis of all the resultant sequences, it was observed that out of twenty samples only three contained the SNP which was totally absent in controls. Further studies with this SNP needed to be carried with a larger size population for understanding the role of this SNP in myeloid leukemia characterisation, symptoms, outcomes and also diagnosis of the disease.

5. CONCLUSION

In conclusion it is seen that three samples out of 20 diseased were having the SNP were as the others were normal in spite of being diseased. All the normal samples were neutral for the SNP. These results can depict that the SNP has some unrevealed relation with the disease as it was totally absent in the normal samples. However all the diseased samples did not show the SNP, thus there must be some criteria by which the absence of SNP still causes the disease? The study can be concluded by increasing the sample size. This can lay a foundation for the development of a diagnostic method based on the detection of the SNP in the gene samples.

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