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Study Polymorphism of *MET* Gene in Cancer Iraqi Patients (AML)

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ABSTRACT

Many genes are mutated in acute myeloid leukemia patients, one of them is Met gene. the aim study was to study the single nucleotide polymorphisms in one loci of met gene in a sample of Iraqi patients. We diagnosed the polymorphism and compared the results with heathy volunteers' gene. A set of forward and reverse primers were used to detect and amplificated met gene by PCR technique. The allele frequency then detected by Restriction fragment length polymorphism (RFLP) for patients and control healthy groups. Non-significant results were found in the recurrence of homozygous AA allele of met gene in AML patients which was 75.67 % while in healthy volunteers was 86%, but the heterozygotes AG of met gene was 24.32 % in AML patients and 14 % in healthy group. The finding of our study show that heterozygotes AG is more related with patients than control group and it occurs more frequently in female patients than male. while homozygous AA appears more frequent in healthy control than the patients and more frequent in male than female.

Keywords: *AML, MET gene, RFLP, polymorphism*

1. INTRODUCTION

MET gene located on chromosome seven translated to many proteins formed the enzyme tyrosine kinase receptor which is product proto oncogene. Oncogene play a vital role in human carcinogenesis[1,2]. Mutation and polymorphism of MET gene found in leukemia and many types of cancer[3] . mutation occur by exogenous factor or endogenous factor that alter the cell cycle and develop malignancy[4,5,6]. Many studies found that MET gene is altered in leukemia and many types of cancer. Acute myeloid leukemia is one of cancer types which related with blood cells abnormalities. the met gene expressed protein conform oncogenes play an important role in the initiation and development of cancer, and in some types mediates the migration, proliferation and invasion of caner cells[7,8]. Although resent studied proposed many new promising therapies against leukemia [9,10,11] , cancers are still one of danger disease having the highest rate of mortality and morbidity[12,13] and still consider as great challenge . therefore, further molecular assay and techniques for exploration, diagnosis and illustration for early diagnosis and treatment are very essential needed in orded to reduce mortality and improve patients treatment and survival [14,15,16]. Gene therapy is one of the most promising methods to achieve fewer number pf patients in the world [17,18].

2. MATERIALS AND METHODS

Methods

Subjects:

Our study involved Seventy four patients who diagnosed with Acute myeloid leukemia (AML) , thirty three (33) of them were male and the rest (41) were female attended to Medical City Hospital ,and fifty healthy volunteer as control (25 female and 25 male). with an age range of (16–73) year .

Sample collection:

Five ml of blood were taken from the vein of each patient and the volunteer in our study. Then the blood was transferred in EDTA tubes to prepare for DNA extraction.

DNA extraction

Genomic DNA was extracted from whole blood samples of the

patients and the controls using Geneaid kit by following the procedure of the kit.

Gel electrophoresis:

After the extraction was complete, agarose gel electrophoresis was depend to confirm the presence of DNA .To prepare 1% agarose gel ,1gm was dissolved and boiled in 100 ml of 1x agarose powder and for 2% agarose gel ,2gm was dissolved and boiled in 100 ml of 1x agarose powder , left to cool at fifty Silesian and then five µl of Ethedium bromide was added to it and poured on preparing tray. Comb was removed after hardening of agarose leaving wells. Loaded every well with ten µl (seven µl of sample and three µl loading dye) and in one well standard marker DNA was loaded. electrophoreses run at five volt/cm of the gel. Agarose was removed from the tank and visualized with the aid of UV transilluminator and photographed.

Polymerase chain reaction (PCR):

PCR amplification of Met gene sequences was performed using following primer pairs (F: 5'-50-GCCTGGTGGTCATCGACTC-3; reverse,5'-ACAGGGCTCTGGAAGGCACTGCTCAGCTC ACGCA CC-3[19]which gives PCR product 136 bp. The mixture of PCR contain 1 µL of each primer (forward and reverse) and 5 µL of genomic DNA mixed with 13 µL of Deionized distil water in pcr tube contain lyophilized pcr master mix. PCR program was : first denaturation at ninety four Silesian for seven minutes followed by thirty five cycle with denaturation at ninety four Silesian for one minutes, annealing at fifty seven Silesian for one minutes, extention at seventy two Silesian for one minutes, then followed by a final extension cycle at seventy two Silesian for seven minutes.

Restriction fragment length polymorphism (RFLP):

The polymorphism of met gene was detected by PCR -RFLP as the method of Wang et al(2014) .Ten µL contain five µL of PCR product for met gene and 0.5 µL R.E. and 4.5 µL buffer were mixed and incubated in 37 °C for ten minutes . then the product were run in agarose gel 3% by electrophoresis at 100 voltage for an hour .then visualized under U.V. light.

R.E. digested the PCR product (136bp) at 37 Silesian to fragments with a molecular weight 39 bp and 97bp in case of the homozygous AA met gene genotype, and to fragments with a molecular weight 136 bp in case of the heterozygous AG Met gene genotype in PCR product

Statistical analysis

Analysis of genetic petameters were done using SPSS program v 20

3. RESULTS AND DISCUSSION:

DNA extraction was done for 124 blood sample (74 sample of patients and 50 healthy volunteers serve as control). Using specific primers for met gene

Conventional PCR was used to amplification of met gene. The product gene fragment was 136bp (figure 1)

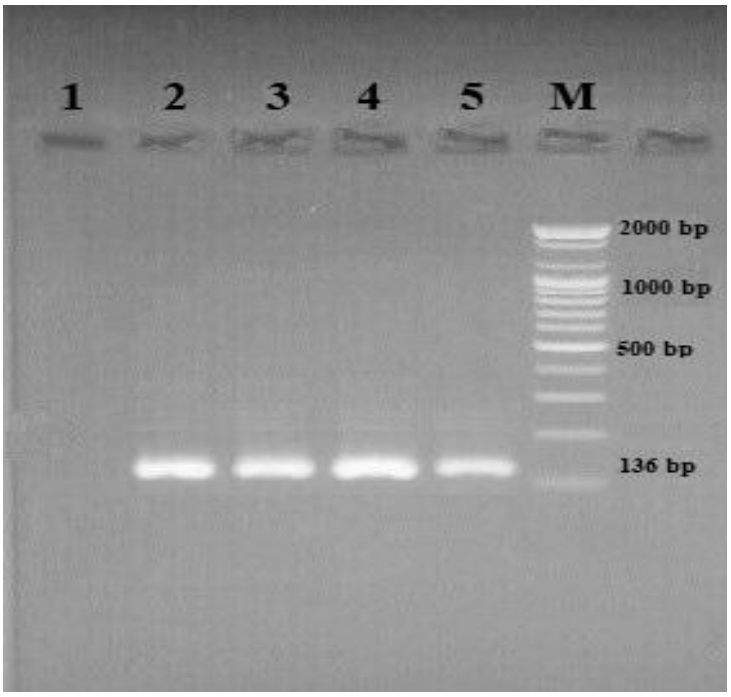

Fig 1 :Gel electrophoresis of met gene PCR product of , lane 1 is negative control, lane 2 to 5 is positive product and M : is 100 bp DNA marker , (2% agarose, , 5V/Cm, 2hr.) visualized under U.V. light.

Restriction fragments length polymorphism

RFLP technique was done for met gene product136 bp and digested it to product 136bp in case of the heterozygous AG and to fragments with a molecular weight 97,39 pb in case of the homozygous AA met gene (figure 2)

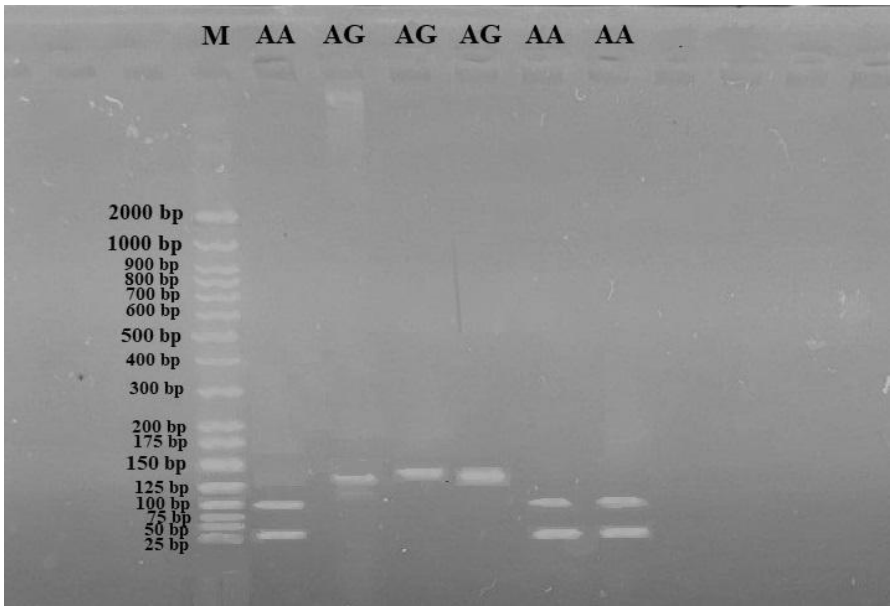

Fig 2:Gel electrophoresis of Restriction fragments length polymorphism of met gene , M : molecular marker, AA : homozygous fragments with a molecular weight 97,39 pb, AG : heterozygous fragments with a molecular weight 136bp .(2% agarose, , 5V/Cm, 2hr.) visualized under U.V. light.

Our study find that the homozygous AA of met gene was repeated with lower ratio in patients (75.67 %) than control group(86%) (table 1) and appear to found in patients male (30) more than patients female (26).while homozygous AA found in control male (23) more than control female (20) (figure 3).

The genotype of MET in AML patients and control shown in table 1

Table 1: The genotype of MET in AML patients and control

genotype	**AML patients**	**Healthy control**
	N. (%)	**N. (%)**
AA	56 (75.67%)	43 (86 %)
GG	0 (0 %)	0 (0 %)
AG	18 (24.32%)	7 (14 %)
Total	74 (100%)	50 (100 %)
P - value	0.018	
Significant at P≤0.01		

While the ratio of heterozygous AG of met gene was repeated in patients (24%) more than control (14%) , appear to found in patients male(3) less than patients female (15) , and in control female (5) more than control male (2)

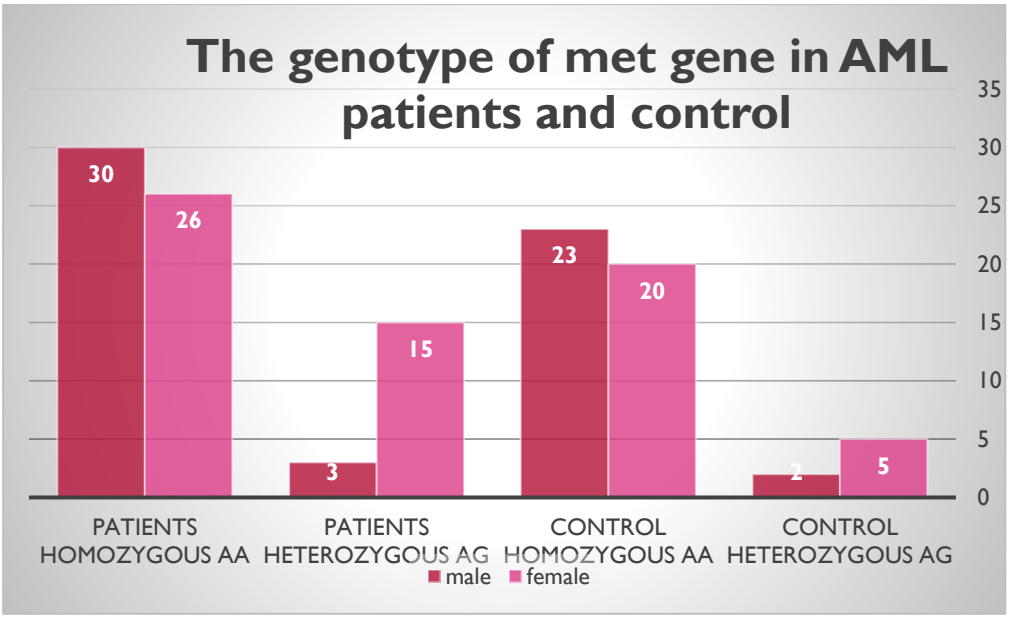

Fig3 : the distribution of genotype homozygous AA and heterozygous AG of met gene in AML patients and control.

4. DISCUSSION

Given the importance of genetic gengs in many cancer disease , many studies have been conducted about this subject[20, 21, 22]. Many of these studies agreed with the results obtained about the occurrence of homozygous AA and heterozygous AG in patients met gene [23,24,25], kury and his colleagues found that the AG allele is repeated in 83% of patients with cancer [26]. although the treatment of AML has improved and updated in the recent years , the AML patients develop diseases that is refractory to the amount and lot of chemotherapy [26, 27]. Genotype profile offer a means to get knowledge and discover the altering in the genome and understood the effect of mediated molecule in developing cancer [29] . Met gene interact with many type of human cancer by different mechanisms,

Studies in different countries noticed that Mutations in the *MET* gene have been found in an inherited cases called hereditary papillary renal carcinoma (HPRC) [30,31]. These Patients with HPRC have an increased risk of a type of kidney cancer called papillary renal carcinoma. *MET* gene mutations involved in liver cancer and many other types of cancer like head and neck cancer [32,33,34,]. AML patients have mutant MET gene with more than one mutation in many cases [35,36], single nucleotide mutation can alter the amino acid code lead to alter in protein code and gene expression[37].

5. CONCLUSION

AML patients have many mutated genes in there genome , MET gene is one of important gene play a vital role in AML cases .our finding lead us to conclude that heterozygotes AG is more related with patients than control group and it occurs more frequently in female patients than male. while homozygous AA appears more frequent in healthy control than the patients and more frequent in male than female.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

[1] Schmidt L, Duh FM, Chen F, et al. Germline and somatic mutations in the tyrosine kinase domain of the Met proto-oncogene in papillary renal carcinomas. Nat Genet 1997;16:68-73.

[2] Agarwal S, Zerillo C, Kolmakova J, Christensen JG, Harris LN, Rimm DL, Digiovanna MP, Stern DF. Association of constitutively activated hepatocyte growth factor receptor (met) with resistance to a dual EGFR/Her2 inhibitor in non-small-cell lung cancer cells. Br J Cancer. 2009;100:941–9

[3] Wu C, Zhuang Y, Jiang S, Liu S, Zhou J, Wu J, Teng Y, Xia B, Wang R, Zou X. Interaction between Wnt/beta-catenin pathway and microRNAs regulates epithelial-mesenchymal transition in gastric cancer (review). Int J Oncol. 2016;48:2236–46

[4] Jeffers M, Fiscella M, Webb CP, et al. The mutationally activated Met receptor mediates motility and metastasis. Proc Natl Acad Sci U S A 1998;95:14417-22.

[5] Benvenuti S, Lazzari L, Arnesano A, Li Chiavi G, Gentile A, Comoglio PM. Ron kinase transphosphorylation sustains MET oncogene addiction. Cancer Res. 2011;71:1945–55.

[6] Wang R, Ferrell LD, Faouzi S, et al. Activation of the Met receptor by cell attachment induces and sustains hepatocellular carcinomas in transgenic mice. J Cell Biol 2001;153:1023-34.

[7] Jeffers MF, Vande Woude GF. Activating mutations in the Met receptor overcome the requirement for autophosphorylation of tyrosines crucial for wild type signaling. Oncogene 1999;18:5120-25.

[8] Bahrami A, Shahidsales S, Khazaei M, Ghayour-Mobarhan M, Maftouh M, Hassanian SM, Avan A. c-Met as a potential target for the treatment of gastrointestinal cancer: current status and future perspectives. J Cell Physiol. 2017;232:2657–73

[9] Cecchi F, Rabe DC, Bottaro DP. Targeting the HGF/met signalling pathway in cancer. Eur J Cancer. 2010;46:1260–70

[10] Graveel CR, DeGroot JD, Su Y, et al. Met induces diverse mammary carcinomas in mice and is associated with human basal breast cancer. Proc Natl Acad Sci U S A 2009;106:12909-14.

[11] Graveel CR, DeGroot JD, Sigler RE, et al. Germline met mutations in mice reveal mutation- and background-associated differences in tumor profiles. PLoS One 2010;5:e13586.

[12] Park WS, Dong SM, Kim SY, et al. Somatic mutations in the kinase domain of the Met/hepatocyte growth factor receptor gene in childhood hepatocellular carcinomas. Cancer Res 1999;59:307-10.

[13] Tuynman JB, Vermeulen L, Boon EM, Kemper K, Zwinderman AH, Peppelenbosch MP, Richel DJ. Cyclooxygenase-2 inhibition inhibits cMet kinase activity and Wnt activity in colon cancer. Cancer Res. 2008; 68:1213–20.

[14] Wu C, Zhuang Y, Jiang S, Liu S, Zhou J, Wu J, Teng Y, Xia B, Wang R, Zou X. Interaction between Wnt/beta-catenin pathway and microRNAs regulates epithelial-mesenchymal transition in gastric cancer (review). Int J Oncol. 2016;48:2236–46.

[15] Lee JH, Han SU, Cho H, et al. A novel germ line juxtamembrane Met mutation in human gastric cancer. Oncogene 2000;19:4947-53.

[16] Ghadjar P, Blank-Liss W, Simcock M, et al. MET Y1253D-activating point mutation and development of distant metastasis in advanced head and neck cancers. Clin Exp Metastasis 2009;26:809-15.

[17] Liu S, Meric-Bernstam F, Parinyanitikul N, et al. Functional consequence of the MET-T1010I polymorphism in breast cancer. Oncotarget 2015;6:2604-14.

[18] de Melo Gagliato D, Jardim DL, Falchook G, et al. Analysis of MET genetic aberrations in patients with breast cancer at MD Anderson Phase I unit. Clin Breast Cancer 2014;14:468-74.

[19] Vigna E, Comoglio PM. Targeting the oncogenic Met receptor by antibodies and gene therapy. Oncogene 2015;34:1883-9.

[20] Berthou S, Aebersold DM, Schmidt LS, et al. The Met kinase inhibitor SU11274 exhibits a selective inhibition pattern toward different receptor mutated variants. Oncogene 2014;23:5387-93.

[21] Ma PC, Jagadeeswaran R, Jagadeesh S, et al. Functional expression and mutations of c-Met and its therapeutic inhibition with SU11274 and small interfering RNA in non-small cell lung cancer. Cancer Res 2005;65:1479-88.

[22] Kong-Beltran M, Stamos J, Wickramasinghe D. The Sema domain of Met is necessary for receptor dimerization and activation. Cancer Cell 2004;6:75-84.

[23] Mak HH, Peschard P, Lin T, et al. Oncogenic activation of the Met receptor tyrosine kinase fusion protein, Tpr-Met, involves exclusion from the endocytic degradative pathway. Oncogene 2007;26:7213-21.

[24] Heist RS, Shim HS, Gingipally S, et al. MET Exon 14 Skipping in Non-Small Cell Lung Cancer. Oncologist 2016;21:481-6.

[25] Schrock AB, Frampton GM, Suh J, et al. Characterization of 298 Patients with Lung Cancer Harboring MET Exon 14 Skipping Alterations. J Thorac Oncol 2016;11:1493-502.

[26] Jorge SE, Schulman S, Freed JA, et al. Responses to the multitargeted MET/ALK/ROS1 inhibitor crizotinib and co-occurring mutations in lung adenocarcinomas with MET amplification or MET exon 14 skipping mutation. Lung Cancer 2015;90:369-74

[27] Rosell R, Moran T, Queralt C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009;361:958-67.

[28] Bahcall M, Sim T, Paweletz CP, et al. Acquired METD1228V Mutation and Resistance to MET Inhibition in

Lung Cancer. Cancer Discov 2016;6:1334-41.

[29] Heist RS, Sequist LV, Borger D, et al. Acquired Resistance to Crizotinib in NSCLC with MET Exon 14 Skipping. J Thorac Oncol 2016;11:1242-5 Paik PK, Drilon A, Fan PD, et al. Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. Cancer Discov 2015;5:842-9.

[30] Tang C, Jardim DL, Falchook GS, et al. MET nucleotide variations and amplification in advanced ovarian cancer: characteristics and outcomes with c-Met inhibitors. Oncoscience 2013;1:5-13.

[31] Zimmer Y, Vaseva AV, Medova M, et al. Differential inhibition sensitivities of MET mutants to the small molecule inhibitor SU11274. Cancer Lett 2010;289:228-36.

[32] Medova M, Pochon B, Streit B, et al. The novel ATP-competitive inhibitor of the MET hepatocyte growth factor receptor EMD1214063 displays inhibitory activity against selected MET-mutated variants. Molecular cancer therapeutics 2013;12:2415-24.

[33] Previdi S, Abbadessa G, Dalo F, France DS, Broggini M. Breast cancer-derived bone metastasis can be effectively reduced through specific c-MET inhibitor tivantinib (ARQ 197) and shRNA c-MET knockdown. Mol Cancer Ther. 2012; 11:214–23.

[34] Kaposi-Novak P, Lee JS, Gomez-Quiroz L, Coulouarn C, Factor VM, Thorgeirsson SS. Met-regulated expression signature defines a subset of human hepatocellular carcinomas with poor prognosis and aggressive phenotype. J Clin Invest. 2006;116:1582–95

[35] You H, Ding W, Dang H, Jiang Y, Rountree CB. c-Met represents a potential therapeutic target for personalized treatment in hepatocellular carcinoma. Hepatology. 2011;54:879–89.

[36] Gaudino G, Follenzi A, Naldini L, Collesi C, Santoro M, Gallo KA, Godowski PJ, Comoglio PM. RON is a heterodimeric tyrosine kinase receptor activated by the HGF homologue MSP. EMBO J. 1994;13:3524–32.

[37] Raghav KP, Gonzalez-Angulo AM, Blumenschein GR Jr. Role of HGF/MET axis in resistance of lung cancer to contemporary management. Transl Lung Cancer Res. 2012;1:179–93.