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Targeted next generation sequencing provides insight for the genetic alterations in liquid biopsy of Egyptian brain tumor patients

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Abstract

Background: Glioblastoma (GBM) is the commonest primary malignant cerebral tumor in adults. Detection of genetic mutations in liquid biopsy is endorsed rapidly throughout several solid neoplasms but still limited in GBM. Our study provides insight for the genetic alterations in liquid biopsy of the newly diagnosed GBM patients using next generation sequencing technology together with identification of the microsatellite instability (MSI) status in those patients.

Results: Eighteen variants detected in 15 genes which were (4, 12 and 2) missense, coding silent and intronic mutations, respectively. The 4 substitution–missense mutations were as follows: Drug responsive *TP53* (p.Pro72Arg) variant was detected in 6 patients (85.7%). *KDR* (p.Gln472His) variant was noted in 4 patients (57.1%) as a result of substitution at c.1416A > T. Two patients revealed *KIT* (p.Met541Leu) variant which result from substitution at c.1621A > C. Only one patient showed mutation in *JAK3* gene which was (p.Val718Leu) variant resulting from c.2152G > C substitution. Regarding MSI status, four cases (57.1%) were MSI-Low and three cases (42.9%) were MSI-High.

Conclusions: This study identifies the molecular landscape and microsatellite instability alternations in Egyptian brain tumor patients, which may have an important role in improving the outcome, survival and may help in evolving a characteristic individual therapy.

Keywords: Glioblastoma multiforme, Next generation sequencing, Activating mutations

Background

Glioblastoma (GBM) is very aggressive and has a median of 12- to 15-month survival with less than 5% of 5 year survival [1]. Patients had a highly different therapeutic response and rates of survival which could be due to tumor heterogeneity [2]. Clinical, pathological examination and imaging techniques are the standard techniques for GBM diagnosis. Invasive tissue biopsy procedure has many risks to those patients, as affecting neurological functions, hemorrhage, etc. [3], with some tumors may be inaccessible due to their location or close to risk organ [4]. Also, imaging techniques cannot discriminate pseudo- and true progression after treatment to prevent unnecessary operations and further useless treatment [5]. Therefore, the need appears for more simple techniques to assess biomarkers from non-surgical samples. Isolation of circulating tumor DNA (ctDNA) from blood has a number of benefits such as decreasing invasive damages and obstacles of getting sufficient tumor tissues. Also, blood sampling is attainable and easy to reiterate when needed which provides a persuasive and achievable

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method to estimate the characteristics of cerebral tumors [6]. However, few presence of ctDNA in blood is a significant challenge for tumor biomarker testing availability and its translation clinically [7]. Incorporation of histological and genetic evaluation is recommended in GBM, with many genes involved such as isocitrate dehydrogenase 1 and 2 mutations, 1p/19q chromosomal codeletion and point mutations in tumor protein 53, etc. [8]. Using next generation sequencing (NGS) in estimation of genetic alternations in liquid biopsy has been grown rapidly across several solid tumors [9] but still limited in GBM especially in Egypt. Many studies revealed mutations in blood of GBM patients such as Piccioni et al. [10] who has used NGS in ctDNA analysis of advanced glioblastoma patients and has observed mutations in TP53, PDGFRA and NF1 genes, etc. Another study had 33 GBM patients showed mutations in *TP53, EGFR and MET* genes, etc. [11].

Study objectives

Our study provides insight for the genetic alterations in liquid biopsy of the newly diagnosed GBM patients using targeted next generation sequencing technology together with identification of the microsatellite instability (MSI) status in those patients.

Methods

Participants and sample preparation

This pilot prospective study included 7 newly diagnosed brain tumor patients and was performed from Dec. 2019 to Jun. 2020. DNA was isolated from blood samples by QIAamp® DNA Mini kit—Catalogue Number ID: 51304 as stated by the manufacturer guidance. Concentration, quality and amplifiability of the isolated DNA samples have been tested before further processing [12].

Sequencing and data analysis

Preparation of the libraries was done by Illumina AmpliSeq™ Cancer Hotspot Panel v2, Catalogue Number: 20019161 detecting 50 genetic mutations. Libraries were examined by 2100 Bioanalyzer instrument using DNA 1000 kit—Catalogue Code: 5067-1504 with the anticipated PCR yield 186–277 bp. Patients' libraries were combined to reach a final sequencing library which ran using MiSeqDx system with read length of 2×150 bp and approximately 17 h to finalize the run [9]. Checking each run quality was done by determining specifications depending on PhiX libraries that provide a cluster density of 865–965 k/mm^2 clusters passing filter for v2 technology, as well as, run's quality score is evaluated. The percentage of bases more than the Q30 is averaged over the whole run with a quality score for v2 technology more than 80% bases higher than the Q30 on 2×150 bp. Sequence reads was aligned to the Genome Reference Consortium Human Build 37 (GRCh37).

Detection of microsatellite instability (MSI) in studied patients

Mononucleotide markers were recommended in the detection of MSI. Thus, we identified 3 mononucleotide markers: BAT25, BAT26 and NR27, according to manufacturer protocol and data were analyzed using Agilent 2100 Bioanalyzer system [12].

Results

Seven patients were included with 6/7 (85.7%) patients were males. The median patient age was 50 years (range 23–58). Right-sided tumor site was common among our patients 5/7 (71.4%). By MRI brain scan, the median size of the tumor was 5 cm (4–6 cm). The clinicopathological features of our patients are described in Table 1. Variant allele frequency (VAF) of each variant (Table 2) and primary analysis revealed 28 mutations (Table 3). Four variants out of 28 were not found in Catalogue

Table 1 Clinicopathological features of studied population

Patient's features	Number; 7
Age in year	
Median	50
Mean ± SD	46.3 ± 12.4
Range	23–58
Gender	
Males	6/7 (85.7%)
Females	1/7 (14.3%)
Complain	
Convulsion	1/7 (14.3%)
Headache	4/7 (57.1%)
Limb paresis	2/7(28.6%)
Histopathological diagnosis	
Glioblastoma grade IV	6/7 (85.7%)
Astrocytoma grade II	1/7 (14.3%)
Side	
Right	5/7 (71.4%)
Left	1/7 (14.3%)
Bilateral	1/7 (14.3%)
Site	
Fronto-temporal	1/7 (14.3%)
Fronto-parietal	2/7(28.6%)
Tempro-parietal	2/7(28.6%)
Temporal	1/7 (14.3%)
Frontal	1/7 (14.3%)
Diameter	
≥ 5 cm	3/7 (42.9%)
< 5 cm	4/7 (57.1%)

Table 2 Assessment of variant allele frequency and MSI status

Variant	No. of patients	P1 GBM grade IV	P2 GBM grade IV	P3 GBM grade IV	P4 GBM grade IV	P5 Astrocytoma grade II	P6 GBM grade IV	P7 GBM grade IV
FLT3-intronic mutation	5		0.47		0.99	1	0.49	0.51
SMARCB1-intronic mutation	2	0.49					1	
FGFR3 p.Thr653 = -coding silent mutation	7	0.99	0.99	1	0.99	1	0.99	0.99
HRAS p.His27 = -coding silent mutation	2	0.48		0.51				
RET p.Leu769 = -coding silent mutation	7	0.47	1	1	0.51	1	1	0.50
RET p.Ser904 = -coding silent mutation	4	0.53	0.50			0.49		0.49
PDGFRA p.Val824 = -coding silent mutation	3	0.50			0.51		0.49	
PDGFRA p.Pro567 = -coding silent mutation	7	1	0.99	1	0.99	1	1	0.99
MET p.Ser178 = -coding silent mutation	2		0.50			0.48		
MET p.Ile377 = -coding silent mutation	1		0.47					
CDKN2A p.Arg58 = -coding silent mutation	1					0.51		
EGFR p.Gln787 = -coding silent mutation	6	0.99	0.48		0.99	0.99	1	0.51
APC p.Thr1493 = -coding silent mutation	4	0.99			0.48	0.99		0.47
IDH1 p.Gly105 = -coding silent mutation	1		0.49					
KIT p.Met541Leu—missense mutation	2			0.48		0.47		
TP53 p.Pro72Arg—missense mutation	6		0.46	0.50	0.52	1	0.51	0.99
KDR p.Gln472His—missense mutation	4			0.51		0.16	0.47	0.49
JAK3 p.Val718Leu—missense mutation	1							0.51
MSI status	7	MSI-L	MSI-HI	MSI-L	MSI-HI	MSI-L	MSI-L	MSI-HI

GMB glioblastoma, *MSI-L* microsatellite instability-low, *MSI-HI* microsatellite instability-high

of Somatic Mutations in Cancer (COSMIC) database with 5 non-coding variants were noted in the intron of a transcript and only 1 variant was a SNP in COSMIC database. Across 15 genes, there were (4, 12 and 2) missense, coding silent and intronic mutations, respectively (Fig. 1). Searching in ClinVar database, 13/18 was benign mutations, 1 variant has conflicting interpretations of pathogenicity, 3 mutations were not recorded and only 1 variant was drug responsive one. Regarding missense variants, Tumor protein TP53 (*TP53* p.Pro72Arg) was detected in 6 patients (85.7%) which was a drug response mutation resulted from c.215C > G. Mutation in Kinase Insert Domain Receptor (*KDR*) gene was found in 4 patients (57.1%); 3 patients were glioblastoma multiforme grade IV and one patient was astrocytoma grade II. This mutation was p.(Gln472His) resulting from c.1416A > T and was not recorded in ClinVar. Two patients had mutation in KIT Proto-Oncogene, Receptor Tyrosine Kinase (*KIT*) gene. It was p.(Met541Leu) variant which results due to c.1621A > C and was known as a benign/likely benign mutation in ClinVar. Only one patient revealed mutation in Janus Kinase 3 (*JAK3*) gene which was p.(Val718Leu) variant resulting from c.2152G > C and recorded in ClinVar as conflicting interpretations of pathogenicity, likely benign or uncertain significance variant. Benign coding silent variants were noticed in the

Table 3 Activating mutations detected and MSI status in the studied population

Gene	Variant	Substitution	dbSNP ID	HGVSc	HGVSp	Number of cases	In silico predictions		FATHMM prediction	ClinVar	COSMIC ID	MSI result (number of cases)
							Sift	PolyPhen				
KIT	A > C	Missense	rs3822214	c.1621A > C	p.(Met541Leu)	2	Tolerated (0.37)	Benign (0.005)	Pathogenic (score 0.74)	Benign/likely benign	COSM28026	MSI -L (2)
TP53	C > G	Missense	rs1042522	c.215C > G	p.(Pro72Arg)	6	Tolerated (0.57)	Benign (0.045)	Neutral (score 0.36)	Drug response	COSM250061 COSM3766191	MSI -L (3) MSI- H (3)
KDR	A > T	Missense	rs1870377	c.1416A > T	p.(Gln472His)	4	Tolerated (0.16)	Benign (0.01)	Neutral (score 0.07)	Not recorded	COSM149673	MSI -L (3) MSI -H (1)
	G > A	Intron	rs7692791	c.798 + 54G > A		6				Not recorded	COSN8870412 COSN8870413	MSI -L (3) MSI -H (3)
	INDEL	Intron	rs869246746 rs3214870 rs397772062	c.2615-37dupC		4				Not recorded	COSN17154192	MSI -L (3) MSI -H (1)
JAK3	G > C	Missense	rs146837396	c.2152G > C	p.(Val718Leu)	1	Deleterious (0.03)	Benign (0.025)	Pathogenic (score 0.80)	Conflicting interpretations of pathogenicity; Likely benign; uncertain significance	COSM5946070	MSI -H (1)
IDH1	C > T	Coding silent	rs11554137	c.315C > T	p.(Gly105=)	1			Pathogenic (score 0.85)	Benign	COSM1741220	MSI-H (1)
APC	G > A	Coding silent	rs41115	c.4479G > A	p.(Thr1493=)	4			Neutral (score 0.46)	Benign	COSM3760869	MSI-L (2) MSI -H (2)
EGFR	G > A	Coding silent	rs1050171	c.2361G > A	p.(Gln787=)	6			Pathogenic (score 0.95)	Benign	COSM1451600	MSI -L (3) MSI -H (3)
CDKN2A	A > C	Coding silent	rs201208890	c.174A > C	p.(Arg58=)	1				Benign/likely benign	COSM6495276 COSM6495277	MSI -L (1)
MET	C > T	Coding silent	rs28444388	c.1131C > T	p.(Ile377=)	1			Neutral (score 0.29)	Benign	COSM5020205	MSI -H (1)
	C > T	Coding silent	rs35775721	c.534C > T	p.(Ser178=)	2			Not applicable	Benign	COSM1579024	MSI -L (1) MSI -H (1)
PDGFRA	C > T	Coding silent	rs2228230	c.2472C > T	p.(Val824=)	3			Pathogenic (score 0.88)	Benign	COSM22413	MSI -L (2) MSI -H **(1)**
	A > G	Coding silent	rs1873778	c.1701A > G	p.(Pro567=)	7			Neutral (score 0.02)	Benign	COSM7410554	MSI -L (4) MSI -H (3)
RET	C > G	Coding silent	rs1800863	c.2712C > G	p.(Ser904=)	4			Neutral (score 0.27)	Benign	COSM3751779 COSM3751780	MSI -L (2) MSI -H (2)
	G > T	Coding silent	rs1800861	c.2307G > T	p.(Leu769=)	7			Pathogenic (score 0.79)	Benign	COSM4418405 COSM4418406	MSI -L (4) MSI -H (3)

Table 3 (continued)

Gene	Variant	Substitution	dbSNP ID	HGVSc	HGVSp	Number of cases	In silico predictions		FATHMM prediction	ClinVar	COSMIC ID	MSI result (number of cases)
							Sift	PolyPhen				
HRAS	T > C	Coding silent	rs12628	c.81T > C	p.(His27 =)	2			Neutral (score 0.07)	Benign	COSM249860 COSM3752426	MSI -L (2)
FGFR3	G > A	Coding silent	rs7688609	c.1959G > A	p.(Thr653 =)	7			Pathogenic (score 0.70)	Not recorded	COSM7410552	MSI -L (4) MSI -H (3)
FLT3	T > C	Intronic	rs2491231	c.1310-3T > C		5			Neutral (score 0.02)	Not recorded	COSM3999060	MSI -L (2) MSI -H (3)
SMARCB1	G > A	Intronic	rs5030613	c.1119-41G > A		2			Neutral (score 0.03)	Benign	COSM1090 COSN17135779	MSI -L (2)
PIK3CA	A > G	Intron	rs3729674	c.352 + 40A > G		5				Uncertain significance	COSN26959779 COSN26959780	MSI -L (3) MSI -H (2)
STK11	T > C	Intron	rs2075606	c.465-51T > C		3				Uncertain significance	COSN6666958	MSI -L (2) MSI -H (1)
ERBB4	A > G	Intron	rs839541	c.421 + 58A > G		4				Not recorded	COSN19690034 COSN27007111	MSI -L (1) MSI -H (3)
	INDEL	Splice region Intron	rs67894136 rs397987661	c.884-7delT		7				Not recorded		MSI -L (4) MSI -H (3)
	INDEL	Splice region Intron	rs748883732	c.884-8_884-7delTT		2				Not recorded		MSI -L (1) MSI -H (1)
NPM1	INDEL	Intron	rs397792554 rs34323200	c.847-5delT		7				Not recorded		MSI -L (4) MSI -H (3)
CSF1R	MNV	3-prime UTR	rs386693509	c.*35_*36delCAinsTC		7				Uncertain significance		MSI -L (4) MSI -H (3)

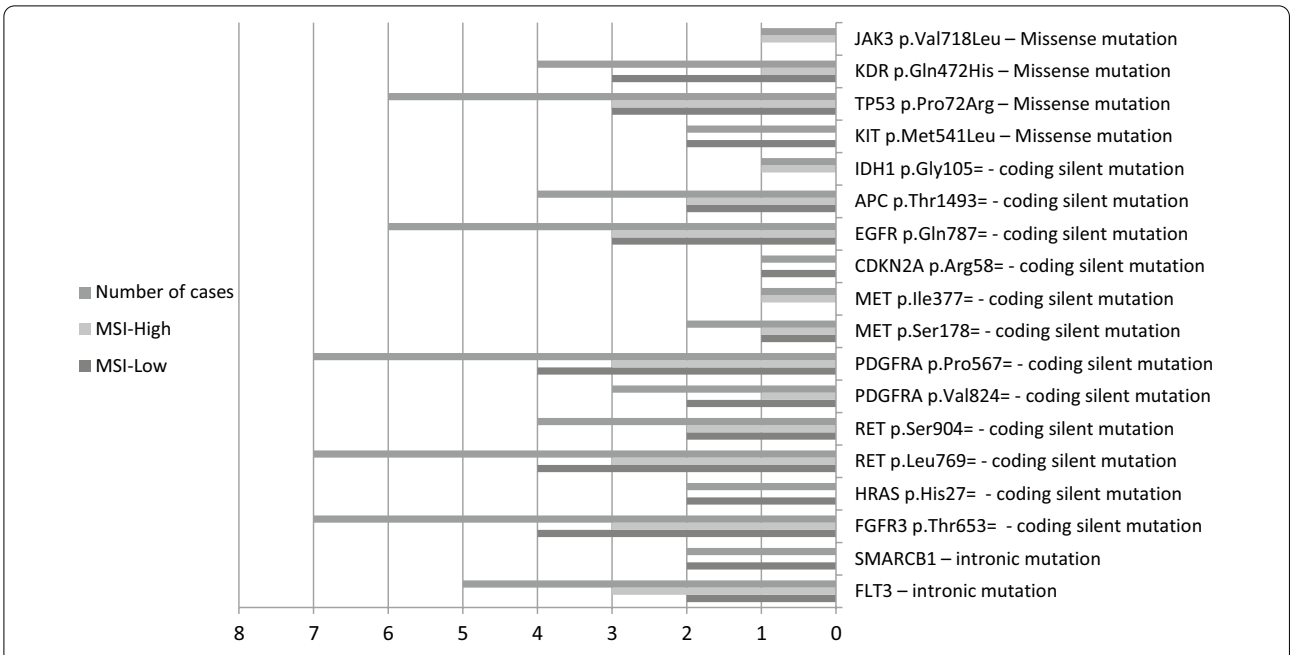

Fig. 1 Different studied activating mutations and MSI status in GBM patients

following genes: Isocitrate dehydrogenase (NADP(+)) 1 (*IDH1*), APC Regulator of WNT Signaling Pathway (*APC*), Epidermal Growth Factor Receptor (*EGFR*), Cyclin Dependent Kinase Inhibitor 2A (*CDKN2A*), HRas Proto-Oncogene, GTPase (*HRAS*). These mutations were p.Gly105=, p.Thr1493=, p.Gln787=, p.Arg58=, and p.His27=, respectively, which result from c.315C > T, c.4479G > A, c.2361G > A, c.174A > C and c.81T > C, respectively. MET Proto-Oncogene, Receptor Tyrosine Kinase (*MET*) gene mutation showed 2 benign variants p.(Ile377=) and p.(Ser178=) due to c.1131C > T and c.534C > T, respectively. Two benign variants were detected in platelet-derived growth factor receptor A (PDGFRA) gene, p.Val824= and p.Pro567= which result from c.2472C > T, and c.1701A > G, respectively. Ret Proto-Oncogene (*RET*) gene revealed 2 benign variants, p.Ser904 = due to c.2712C > G and p.Leu769 = due to c.2307G > T. Fibroblast growth factor receptor 3 (*FGFR3*) gene revealed p.(Thr653=) due to c.1959G > A which is not recorded in ClinVar. Two intronic mutations were noticed in Fms-Related Receptor Tyrosine Kinase 3 (*FLT3*) gene in 5 patients due to c.1310-3T > C and SWI/SNF-Related, Matrix-Associated, Actin-Dependent Regulator of Chromatin, Subfamily B, Member 1 (*SMARCB1*) gene in 2 cases due to c.1119-41G > A. As regards MSI status, 4/7 (57.1%) cases had MSI-Low and 3/7 (42.9%) cases had MSI-high (Fig. 1).

Discussion

Recently, our information about the genetic features of cerebral tumors has raised dramatically by using next generation sequencing platforms [13]. Liquid biopsy has been widely used in solid tumors to identify driver mutations, but is still limited in glioblastoma multiforme (GBM) patients [14]. Our pilot study aimed to assess the activating variants in blood samples of the newly diagnosed GBM using targeted next generation sequencing together with identification of the microsatellite instability (MSI) status. We found the drug responsive variant p.(Pro72Arg) of the tumor suppressor *TP53* gene in 6/7 (85.7%) patients. Previous reports showed that *TP53* is mutated in 29% of the GBM samples and another study showed TP53 mutation in 38% of gliomas, including 23% of primary glioblastomas and 80% of secondary glioblastomas [15, 16]. Other studies showed that *p53* is the commonest mutation noticed in the blood derived ctDNA samples of gliomas [17]. The drug responsive variant p.(Pro72Arg) of the *TP53* gene was found in 47.94% ependymoma grade III and also detected in a young medulloblastoma patient [18]. Kinase Insert Domain Receptor (*KDR*) gene which is a Vascular Endothelial Growth Factor Receptor 2 (*VEGFR2*) gene has a role in tumor initiation and neovascularization [19]. We found *KDR* gene mutation in 4 patients (57.1%) which is p.(Gln472His) as a result of c.1416A > T. *KDR* p.(Gln472His) is a germline variant observed in fifty percent of GBM and forty-seven percent of grade

2–3 astrocytomas [20]. A correlation between *KDR* p.(Gln472His) and risk of glioma has been observed, as unusual angiogenesis may be implicated in primary tumorigenesis [21] with the more angiogenic activity, the worse the survival rate. Thus, GBM patients with the p.(Gln472His) substitution have poor prognosis and this may be related to increases in micro vessel density [22]. Other reports found better survival in positive *KDR* p.(Gln472His) head and neck squamous cell carcinomas [23]. *KIT* mutations have been described in tumor cell proliferation, such as cancer stem cell proliferation, and proliferation of endothelium in gliomas and assisting tumor-related angiogenesis [24]. Here, we observed *KIT* p.(Met541Leu) variant in 2 (28.6%) patients which result from substitution at c.1621A > C. This variant has been described to enhance the receptor affinity to its ligand, stem cell factor (SCF) [25]. Zaman et al. [20] observed *KIT* M514L in 43.75% of both patients of GBM and glioma grade 2–3 and this variant may be used as a marker of aggressiveness which result from mechanisms that do not include regulation of angiogenesis. In our study, only one patient revealed *JAK3* p.(Val718Leu) variant resulting from c.2152G > C substitution. JAK3 is a gene encodes a protein-tyrosine kinase which functions in cytokine receptor-mediated signal transduction and altered in 1.90% of all tumors [26]. As regards MSI status in our GBM patients, 4/7 (57.1%) had MSI-Low and 3/7 (42.9%) had MSI-High. Viana-Pereira et al. [27] found that 13.5% of high-grade glioma samples presented instability, with (< 1%, 12.5% and 86.8%) are MSI-H, MSI-L stable tumors, respectively. Previous study noticed about 27% MSI in 45 pediatric high-grade gliomas using mononucleotide (BAT25 and BAT26) markers [28], another study did not note MSI in 41 cases using (CAT25, BAT25 and BAT26) [29]. Further studies are needed to explore whether liquid biopsy in brain tumor patients could potentially defeat the natural difficulty developed accompanied by the standard tissue biopsy. Larger sample size and longer follow-up period are recommended to compare genetic mutations and MSI status in liquid based versus tissue-based biopsy by targeted next generation sequencing.

Conclusions

Development of noninvasive or minimally invasive approaches to discover and monitor tumors is a major challenge and still limited in our brain tumor patients. This study identifies the molecular landscape and microsatellite instability status in a sample of Egyptian brain tumor patients, which may have an important role in improving the outcome, survival rate and to develop new personalized treatments.

Abbreviations

APC: APC Regulator of WNT Signaling Pathway; CNS: Central nervous system; COSMIC: Catalogue of Somatic Mutations in Cancer; ctDNA: Circulating tumor DNA; EGFR: Epidermal Growth Factor Receptor; FLT3: Fms-Related Receptor Tyrosine Kinase 3; GBM: Glioblastoma multiforme; GRCh37: Genome Reference Consortium Human Build 37; HNSCC: Head and neck squamous cell carcinoma; HRAS: HRas Proto-Oncogene, GTPase; IDH1/2: Isocitrate dehydrogenase (NADP(+)) 1/2; IRB: Institutional Review Board; KDR: Kinase Insert Domain Receptor; MET: MET Proto-Oncogene, Receptor Tyrosine Kinase; MGMT: O6-methylguanine methyltransferase; MSI: Microsatellite instability; NCI: National Cancer Institute; NGS: Next generation sequencing; PDGFRA: Platelet-derived growth factor receptor alpha; RET: Ret Proto-Oncogene; SCF: Stem cell factor; SMARCB1: SWI/SNF-Related, Matrix-Associated, Actin-Dependent Regulator of Chromatin, Subfamily B, Member 1; TP53: Tumor protein TP53; VCF: Variant Call Format; VEGFR2: Vascular Endothelial Growth Factor Receptor 2.

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Authors' contributions

NK and MH elucidated the patient data. HS picked up the clinical data. HK analyzed the genetic data and was the main writer of the manuscript. All authors read and accepted the final manuscript.

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Availability of data and materials

Available upon reasonable request.

Declarations

Ethics approval and consent to participate

The study was accepted by Kasr Al Ainy Clinical Oncology department Institutional Review Board (IRB)-11-2019. Informed consent in a written form was taken from all patients involved in this work.

Consent for publication

This consent was obtained from all patients involved in this work.

Competing interests

No conflict of interest has been declared.

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