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Next-generation sequencing (NGS) detection of PIK3CA somatic mutations in Hepatocellular carcinoma (HCC) Egyptians

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ABSTRACT

Hepatocellular carcinoma (HCC) is the tertiary greatest communal malignant cancer that origins mortality globally. A high prevalence of return means that even while cancer is getting more treatable in its early stages, advanced cases have a bad prognosis. To properly treat HCC, it is now necessary to understand its pathogenic process and its associated genetic abnormalities. The next-generation sequencing (NGS) was used to discover PIK3CA somatic mutations in HCC Egyptian patients. In the present study a unique NGS panel (AmpliSeq) containing the PIK3CA gene was utilized to examine 21 liquid biopsy samples from patients with HCC. The results indicated that over 40 -single-nucleotide-variation (SNV) were recognized in PIK3CA gene with incidence of 85.7%. The changes were dispersed between deleterious, undefined significance, and tolerated deviations, where the preponderance of the changes were the missense variants (68%),synonymous(25%), and (7%) for stop-gained. It was concluded from this research that detection of numerous somatic mutations of PIK3CA can assist in the etiology of HCC.

Keywords: PIK3CA, HCC, Non-Synonymous, Mutation, SNV, NGS.

INTRODUCTION

Hepatocellular carcinoma is considered the 3rd lethal cause in the world depending on its relation with the mortality (Morishita and Masaki, 2015). Although case detection has evolved, due to its incurability to treat and poor prognosis, it has become one of the most prevalent and renowned forms of malignancy (Venook *et al*., 2010). The clear knowledge and good understanding of its pathophysiology is very helpful and potential for an accurate diagnosis and medical cure (Morishita *et al*., 2018).When discussing HCC risk factors, it is vital to emphasize cirrhosis, the advancement of which is the major cause of this form of cancer (Llovet *et al*., 2003; Schuppan and Afdhal, 2008).Cirrhosis is caused by a variety of reasons, including viral infections (HBV, HCV), drinking, non-alcoholic steatohepatitis, and autoimmune hepatitis (El-Serag, 2012).

There are hotspot genetic mutations that play an important role in causing HCC such as CTNNB1, TP53, APC, KRAS, NOTCH1 and PIK3CA, the detection methods of these mutations are highly significant to aid the diagnosis and medical intervention as well as a fair prognosis. PIK3CA is a chromosome 3 regulatory and catalytic subunit. This gene encodes the catalytic subunits that phosphorylate pip2 to pip3. This gene is oncogenic. The PI3K-AKT-mTOR pathway is a master controller of activities involved in

carcinogenesis and tumor preservation (Brown and Toker, 2015).

PI3K phosphorylates (PIP2) and converts it to (PIP3), which attaches to and triggers the AKT (Kudo , 2012)[9]. PTEN, a product of tumor suppressor gene, is opposed to PI3K action; PTEN gene deletion boosts PIP3 levels and triggers AKT, which prevents apoptosis, resulting in tumorformation (Georgescu , 2010; Chalhoub and Baker, 2009). Although the role of PI3KCA mutations in HCC is debated, PI3KCA is identified upstream of AKT.

Next-generation sequencing (NGS) has improved medical diagnostic accuracy and hence therapy efficacy. The present study aims to identify PIK3CA somatic mutations in Egyptian HCC patients using NGS.

PATIENTS AND METHODS

1. Patients:

This study comprised 21 patients with hepatocellular cancer from the Liver National Institute-Menoufia University-Egypt oncology clinic. These patients' genomic targets were compared to 3 healthy people with no malignancies. The study was approved by the Menoufia University Ethics Committee (NLI IRB procedure00232/2020, Dec.2020). Other cancer patients were excluded from this investigation.

2. Methods:

A comprehensive blood count was performed on all HCC patients, as well as a thorough medical and family history evaluation.

2.1 Samples:

Each patient had (1-3) ml EDTA-blood drawn. For cell-free DNA extraction, whole blood genomic DNA was maintained at -80°C in plasma or serum.

2.1 Next generation sequencing:

To extract cell-free DNA from plasma samples, the QIAamp® DSP Virus spin kit Version 1 (QIAGEN, Hilden, Germany) was employed. Thermo-scientific's Gene JET purification kit (K0721) was used to extract genomic DNA. These included Ion AmpliSeq™ tailored NGS panel covering PIK3CA gene (version 2; Thermo Fisher Scientific, Inc.) and Ion AmpliSeq™ HiFi Master Mix (Ion AmpliSeq™ Library kit 2.0, Thermo Fisher Scientific, Inc). The library was then quantified using the ion library TaqMan® Quantitation Kit (Thermo Fisher Scientific, Inc.) as instructed (Morishita *et al*., 2018).

The Ion OneTouch™2 system was used to equip and enhance the templates (Life Technologies). The Ionsphere quality control kit (Thermo Fisher Scientific, Inc) was utilized to ensure that between 10% and 30% of template-positive ISPs were produced (Morishita *et al*., 2018).

After enrichment, the template ISPs were loaded onto Ion 316™ chips, and sequenced as the manufacturer's information using the IonPGM™ Sequencing Hi-Q view kit v2 and PGM™ (Life Technologies) (Morishita *et al*., 2018).

2.3Bioinformatics analysis of data:

Thermo Fisher's Ion reporter server 5.10 was utilized to analyze normal and tumor samples using the ion ampliseq custom panel procedure's default plugin parameters. Torrent Suite was used to match data to Human Genome Version 19 (hg19) (version 3.6.2; Thermo Fisher Scientific, Inc.). The Coverage Analysis plug-in was used (version 3.6; Thermo Fisher Scientific, Inc.). Quality >20, average base coverage >500x reads, allele frequency >10%, and overall uniformity >80% were the cut-offs. The plug-in Variant Caller discovered mutations (version 3.6; Thermo Fisher Scientific, Inc.). The Integrative Genome Viewer (IGV) of the Broad Institute was used to

verify each mutation (www.broadinstitute.org) (Thorvaldsdóttir *et al.*, 2013).

3 Statistical analysis:

The connection between PIK3CA mutations and clinicopathological factors was assessed using the fisher's exact test in SPSS software (version 21). P>0.05 denoted a statistically significant difference (Table 2).

RESULTS

Study population:

It was obvious from data in Table (1) for the investigated 18 males and 3 females in this study that eleven of them were over 62 and 10 were under 62. One HBV positive patient and two non-viral hepatitis patients were involved in the research. The BCLC staged 7 A, 6 B, 6 C, and 2 D patients (Fig. 1).

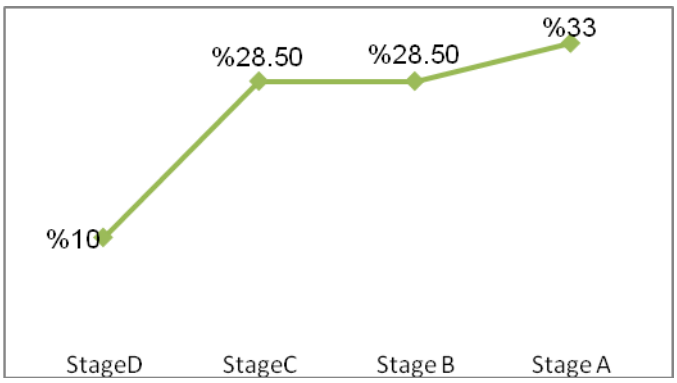

Fig. (1): Classification of HCC patients according to Barcelona clinic liver cancer.

1. PIK3CA mutations' profiling:

In the present study the PIK3CA gene was mutated in 18/21 (85.7%) of samples. When compared to the genomic control, there were 40 SNV mutations, 34 somatic and 6 germlines. SNV somatic mutations were classified as non-synonymous in 19/34(56%), synonymous in 7/34(21%) and unavailable in 8/34 (23%) (Tables 3, 4 and 5). In the VEP analysis of SNV mutations, the count of missense variants was the highest (68%) followed by synonymous (25%) and stop-gained (7%) (Fig. 2).

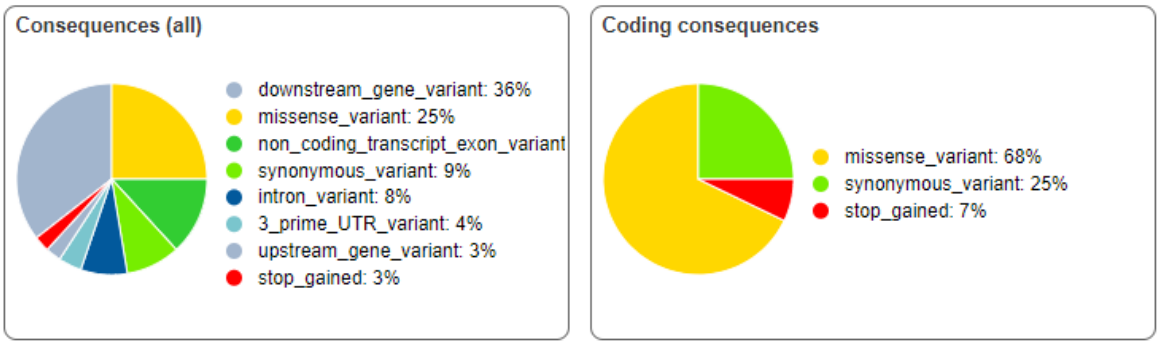

Fig. (2). Summary of PIK3CA somatic mutations among HCC patients

Table (1). Clinical data of HCC Patients and the distribution of PIK3CA somatic mutations among them.

Pt. code	BCLC	Age	Gender	Smoking	HCV	HBV	NBNC	P.S	P.V	No.L	Meta	L.N	C-P	Locus of Mutation	Mutation	E/N
HCC-1	A	63	M	YES	pos	neg	no	0		1			A	chr3:178927912	A>A/G	n
														chr3:178938808	T>C/C	e
														chr3:178938810	T>C/C	n
														chr3:178938813	C>C/G	n
														chr3:178938821	T>T/G	n
														chr3:178952090	GG>GG/TC	-
														chr3:178952091	G>G/C	e
HCC-2	A	67	M	NO	pos	neg	no	0		1			A	chr3:178952215	G>C/C	n
														chr3:178952217	A>C/C	n
														chr3:178927932	T>T/C	n
														chr3:178921504	TT>CC/TG	-
HCC-3	A	55	F	NO	pos	neg	no	0		1			A	chr3:178938870	G>G/A	e
HCC-4	A	59	M	YES	pos	neg	no	0		1			A	chr3:178921471	T>T/G	n
														chr3:178921507	T>T/C	n
														chr3:178927912	A>A/G	n
														chr3:178927932	TTT>TTT/CGA	n
														chr3:178927935	ATA>GCC/GCC	n
														chr3:178927984	CAT>AGC/AGC	-
HCC-5	A	80	M	NO	pos	neg	no	0		1			A	chr3:178952193	A>A/C	n
HCC-6	A	61	M	NO	pos	neg	no	1		1			A	chr3:178952193	A>A/C	n
HCC-7	A	63	M	NO	pos	neg	no	0		2			A	chr3:178927931	T>T/G	n
HCC-8	B	79	M	NO	pos	neg	no	0		3			A		No mutation	-
HCC-9	B	54	M	EX15Y	pos	neg	no	0		multiple	lung	yes	A	chr3:178916875	C>C/T	e
														chr3:178921523	A>A/G	n
														chr3:178938837	T>T/C	n
														chr3:178938877	G>G/A	e
														chr3:178938918	A>A/C	n
														chr3:178938880	G>G/T	n
HCC-10	B	57	M	EX1Y	pos	neg	no	0		multiple			A	chr3:178927931	T>T/G	n
														chr3:178952141	G>A/A	e
HCC-11	B	68	F	NO	pos	neg	no	0		2			A		No mutation	-
HCC-12	B	53	M	NO	pos	neg	no	0		multiple			A	chr3:178927986	T>T/C	e
														chr3:178952193	A>A/C	n
HCC-13	B	50	M	NO	pos	neg	no	0		multiple			A		No muataion	-
HCC-14	C	48	M	NO	pos	neg	no	0		2		yes	A	chr3:178927912	A>A/G	n
														chr3:178927965	T>T/C	n
HCC-15	C	68	M	EX19Y	pos	neg	no	0		3	lung	yes	A	chr3:178921567	A>A/G	e
HCC-16	C	63	M	YES	neg	neg	yes	0	yes	1			A		No mutation	-
HCC-17	C	60	M	YES	neg	neg	yes	1	yes	2			B	chr3:178921500	T>T/G	n
														chr3:178927912	A>A/G	n
HCC-18	C	65	M	NO	pos	neg	no	1		1			B	chr3:178927929	T>T/G	n
														chr3:178936050	T>T/C	e
														chr3:178952193	A>A/C	n
														chr3:178952195	T>T/A	n
														chr3:178952215	G>C/C	n
														chr3:178952217	A>C/C	n
HCC-19	C	67	M	EX13Y	pos	neg	no	0		multiple			B	chr3:178921500	T>T/G	n
HCC-20	D	53	M	NO	neg	neg	yes	0	yes	1			C	chr3:178921502	G>G/T	e
														chr3:178921503	GTTA>GTGA/TTGG	-
														chr3:178921507	T>T/C	n
														chr3:178921508	A>A/T	n
														chr3:178921510	A>A/G	n
														chr3:178921511	TA>CC/CC	-
														chr3:178927912	A>A/G	n
														chr3:178927932	TTT>TTT/CGA	n
														chr3:178927935	ATAA>TGCC/TGCC	-
														chr3:178927984	CAT>AGC/AGC	-
														chr3:178938805	A>A/T	n
														chr3:178938806	G>G/T	e
														chr3:178938810	T>T/C	n
														chr3:178938895	A>A/G	n
														chr3:178952003	G>G/T	e
HCC-21	D	67	M	NO	pos	neg	no	0		3	lung		C	chr3:178927929	T>T/G	n

*pt.code=patient code, *BCLC=Barcelona clinic liver cancer, *HCV=hepatitis C virus, *HBV=hepatitis B virus, *NBNC=none B none C, *P.S=performance status, *P.V=Portal vein, *No.L=Number of lesions, *Meta=metastasis, *L.N=lymph node, *C.P=child pugh, *EX=before*E=existing, *Novel=Novel, *pos=positive, *neg=negative, *M=male, *F=female.

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Table (2). Association between clinicopathological features and representative genetic mutations in the investigated hepatocellular carcinoma patients.

	PIK3CA			
Characteristic	N	Wild-Type	Mutant	P-Value
Sex	21			1.000
Female	2	0	2	
Male	19	3	16	
Age, years				0.586
<62	10	2	8	
≥62	11	1	10	
Viral infection				0.386
HCV	18	1	17	
HBV	0	0	0	
NBNC	3	1	2	
PS				0.386
0	18	2	16	
1	3	1	2	
BCLC				0.943
• A	7	1	6	
• B	6	1	5	
• C	6	1	5	
• D	2	0	2	
Smoking				0.595
YES	4	1	3	
	4	1	3	
NO	13	1	12	
Medical. H				0.812
Bilharziasis	13	2	11	
DM	9	1	8	
HTN	4	0	4	
IHD	1	0	1	
NIL	3	1	2	
metastasis	3	0	3	
L.N	3	0	3	
No of lesions 1 2 3 >3	 9 4 3 5	 1 1 1 0	 8 3 2 5	
P.V	3	1	2	

*pt.code=patient code.*BCLC=Barcelona clinic liver cancer.*HCV=hepatitis C virus.*HBV=hepatitis B virus.*NBNC=none B none C.*P.S=performance status.*P.V=Portal vein.*No.L=Number of lesions.*Meta=

Table (3). Non-synonymous somatic mutations of PIK3CA in HCC patients.

no	Ch_Locus_Allele	Var.alle. freq	Impact	Type	CDs_ Position	AA-changes	Codons	Links	SIFT	Polyphen	AA	E/N
1	3_178916875_C/T	0.04	H	non synonymous	C262T	R88*	Cga/Tga	COSV104566612	-	-	C	E
2	3_178921502_G/T	0.04	mo.t	non synonymous	G984T	W328C	tgG/tgT	**COSV55908849**	Tolerated	Possibly Damaging	G	E
3	3_178921567_A/G	0.04	mo.t	non synonymous	A1049G	D350G	gAc/gGc	rs1553821144, COSV55877939, COSV55937337	Deleterious	Possibly Damaging	A	E
4	3_178938806_G/T	0.04	mo.t	non synonymous	G2048T	R683M	aGg/aTg	-	Deleterious	Probably Damaging	G	E
5	3_178938808_C/C	0.04	-	non synonymous	-	-	-	-	-	-	-	E
6	3_178952003_G/T	0.04	mo.t	non synonymous	G3058T	A1020S	Gca/Tca	COSV55994753 COSM6475717	tolerated	Benign	G	E
7	3_178952091_G/C	0.04	mo.t	non synonymous	G3146C	G1049A	gGt/gCt	COSV55880767, COSV55907597	tolerated	Possibly Damaging	G	E
8	3_178927986_T/C	0.04	L	non synonymous	T1264C	L422L	Ttg/Ctg	rs781513842			-	E
9	3_178952141_A/A	0.08	-	non synonymous	-	-	-	-	-	-	-	E
10	3_178921471_T/G	0.04	mo.t	non synonymous	T953G	M318R	aTg/aGg	-	Tolerated	Benign	T	N
11	3_178921507_T/C	0.08	mo.t	non synonymous	T989C	I330T	aTa/aCa	-	Deleterious	Benign	T	N
12	3_178921510_A/G	0.04	mo.t	non synonymous	A992G	N331S	aAt/aGt	-	Tolerated	Benign	A	N
13	3_178938805_A/T	0.04	mo.t	non synonymous	A2047T	R683W	Agg/Tgg	-	deleterious	Probably Damaging	A	N
14	3_178938821_T/G	0.04	mo.t	non synonymous	T2063G	L688W	tTg/tGg	-	deleterious	Probably Damaging	T	N
15	3_178938880_G/T	0.04	mo.t	non synonymous	G2122T	A708S	Gca/Tca	-	deleterious	Possibly Damaging	G	N
16	3_178938895_A/G	0.04	mo.t	non synonymous	A2137G	I713V	Att/Gtt	-	Tolerated	Benign	A	N
17	3_178927984	0.08	-	non synonymous	-	P421Q		-	-	-		N
18	3_178938918_A/C	0.04	Mot	non synonymous	A2160C	K720N	aaA/aaC	-	deleterious	Possibly Damaging	A	N
19	3_178921500_T/G	0.08	Mot	non synonymous	T982G	W328G	Tgg/Ggg	-	deleterious	Possibly Damaging		N

*no = Case number.*ch = chromosome. *CDs=codings.*AA = amino acid.*SIFT = .*Polyphen = .*E= Existing.*N= novel.*h=high.
*mot = moderate.*rs = reference sequence.*H= high.*L= low.*var.alle.freq = variant allele frequency.

Table (4). Synonymous somatic mutation of PIK3CA in HCC patients.

no	ch_locus_allele	impact	Var.alle.freq	cDNA_position	CDS_position	AAchanges	Codons	Existing_variation	SIFT	PolyPhen
1	3_178938870_G/A	L	0.04	2269	G2112A	R704R	agG/agA	rs201244572	no prediction	no prediction
2	3_178921508_A/T	L	0.04	1147	A990T	I330I	atA/atT	-	no prediction	no prediction
3	3_178921523_A/G	L	0.04	1162	A1005G	R335R	agA/agG	-	no prediction	no prediction
4	3_178938810_C/C	-	0.08	-	-		-	-	no prediction	no prediction
5	3_178938813_C/G	L	0.04	2212	C2055G	G685G	ggC/ggG	-	no prediction	no prediction
6	3_178938837_T/C	L	0.04	2236	T2079C	R693R	cgT/cgC	-	no prediction	no prediction
7	3_178927984	-	0.08	-	-	L422L	-		no prediction	no prediction

*no= Case number.*ch= chromosome. *SIFT= sorting intolerant from tolerant.* PolyPhen: Polymorphism Phenotyping.*L=low

Table (5) non - available somatic mutations of PIK3CA in HCC patients.

no	ch_locus_allele	Var.alle.freq	impact	gene	mutation	cDNA_position	SIFT	PolyPhen
1	3_178927912_A/G	0.2	mo.f	PIK3CA	A>A/G	-	-	-
2	3_178927931_T/G	0.08	mo.f	PIK3CA		-	-	-
3	3_178927932_T/C	0.08	mo.f	PIK3CA		-	-	-
4	3_178927965_T/C	0.04	mo.f	PIK3CA		-	-	-
5	3_178952193_A/C	0.16	mo.f	PIK3CA		3405	-	-
6	3_178952195_T/A	0.04	mo.f	PIK3CA		3407	-	-
7	3_178952215_C/C	0.12	-	-		-	-	-
8	3_178952217_A/C	0.12	mo.f	PIK3CA		3429	-	-

*no= Case number.*ch=chromosome.*mo.f = modifier.*SIFT= sorting intolerant from tolerant.* PolyPhen:Polymorphism Phenotyping.*var.alle.freq = variant allele frequency

3.Non-synonymous mutations:

According to the reading of prediction tools like SIFT (Kumar et al., 2009)[15], and polyphen (Adzhubei et al., 2013; Adzhubei*et al*., 2010)which predicts that some non-synonymous mutations may cause critical changes in protein, a total of 19 non-synonymous variants were recognized, nine of which were reported as presented variants either in single nucleotide polymorphism database (dsSNP) or (COSMIC) as shown in Table (3)**.**

4.Synonymous mutations:

As shown in Table (4) and Figure (3), there were seven synonymous mutations found in the exonic region of the mutant PIK3CA gene.

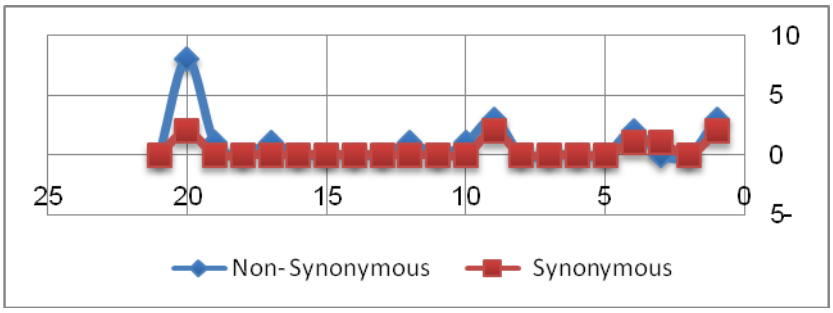

Fig. (3): Distribution of Non-synonymous and synonymous mutations of PIK3CA among HCC patients.

5.Clinical characteristics and genetic alterations:

In HCC, the PI3K/AKT pathway is crucial. In some malignancies, mutant PIK3CA, which encodes the catalytic subunit p110a, enhances AKT pathway activation and cell proliferation. The genetic modifications of PIK3CA were 85.7 percent in the current study, which differs from prior studies on this gene, which showed 4 percent in COSMIC.

DISCUSSION

The accumulation of genetic abnormalities is critical for the appearance and progression of tumors in tissues, and despite the remarkable advancement in technology for examining genetic changes to disease-causing genes, the picture is still not clear enough to explain exactly what happens (Han, 2012).

Among this work, the targeted sequencing was used to look for PIK3CA

genetic variations in a cohort of Egyptian HCC patients. Numerous earlier described and novel genomic variations with known or unknown biological importance were found.

Non-synonymous mutations were often found in PIK3CA variations, and their predicted effects on protein activity raised concerns for HCC development. The oncogene PIK3CA has been associated to a range of cancers, including HCC (Ashktorab *et al*., 2017) by an average of 4% in COSMIC, while the amount varies based on risk variables, sample size, and ethnicity. In the current research the genetic modifications of PIK3CA were 85.7 % which differs which is high and this may be due to the small sample size and ethnicity. The rate of PIK3CA mutation in HCC is contested, with no mutation cases discovered in Japanese research and a high mutation rate of 35.6 percent reported in Korean studies (Kumar et al., 2009).These changes were found to be strongly linked to tumour progression, implying that they could be used to predict HCC formation (Kim *et al*., 2014).

. In the current investigation, the PIK3CA mutation is found to play a minimal impact in HCV-associated hepatocarcinogenesis. Non-synonymous somatic mutations in PIK3CA have been associated to lung, endometrial, colon, stomach, and liver malignancies. Similar observations were given by Aoki*et al*. (2020) and Ashktorab *et al*. (2017).

In the present study, we identified 7/22 non-synonymous mutations in PIK3CA that have a deleterious effect on protein functions and are reported in the COSMIC database, such as (D350G and R683M). The bulk of the non-synonymous mutations in PIK3CA that we found (R683W, L688W, A708S, K720N, and W328G) are not described in COSMIC and warrant additional study because to their possible role in HCC formation.

Synonymous mutations are silent mutations that change a gene's sequence but not the coding protein. Recent study shows that synonymous mutations frequently impact exonic and mRNA splicing patterns, disproving the widely accepted belief that synonymous mutations are quiet (Supek*et al*. , 2014).

A major significance for synonymous SNPs in human disease risk and other complicated aspects has also been revealed in GWAS (Sauna and Kimchi-Sarfaty, 2011). The number of synonymous mutations in PIK3CA may influence HCC risk.

Because all P values in the present study were over 0.05, the fisher's exact test (SPSS) indicated no statistical significance between clinical features and changed gene (Table 2). Our findings included R88* stop-gained mutation in Case 9 who is 54 years old, has not smoked in 15 years, is positive for HCV, negative for HBV, has many lesions in the liver, lymph nodes, and lung metastasis, and other deleterious somatic mutations such as K720N, A708S.Although D350G and R683M are already known to cause harm, new mutations like R683W, L688W, A708S, K720N and W328G need to be studied in a bigger cohort to determine their impact on HCC development.

The cases within this work were classified into subgroups on the basis of Barcelona clinic liver cancer classification (BCLC); with group A representing novices, B representing intermediate instances, and C&D representing advanced cases. In terms of advanced cases, they were (6C, 2 D) 8/21, ranging from 14 to 21 (Table 1).

There were two somatic mutations with no data available at loci (3:178927912 A> A/G, 3:178927965 T> T/C) in Case 14 which was a 48-year-old guy with no smoking, positive for HCV, negative for HBV, two lesion in the liver with lymph nodes, and A child-Pugh. Case 15 It's for a 68-year-old man with HCV, no HBV, three liver lesions, lung metastases with lymph nodes, and child-Pugh A. This patient developed BCLC and had one somatic mutation in PIK3sa (D350G)

According to the COSMIC database, this mutation is non-synonymous, has a moderate effect, and is potentially deleterious.

Case 16 is a 63-year-old guy who smokes heavily (20 cigarettes per day), has portal vein invasion, and one liver lesion. Without a faulty PIK3CA gene, this patient's situation is deemed normal, with other altered genes and causes causing the HCC.

Case 17 is a 60-year-old man smoker with neither HCV or HBV, two liver lesions, no metastasis or lymph node, and a B child-Pugh patient. Specifically, two somatic (W328G, N.A) and one germline (3:178921500 T>T/G, 3:178927912 A>A/G, 3:178936044 A>A/G) mutations were found. W328G is a new non-synonymous somatic mutation that is predicted to be detrimental and perhaps harmful.

Case 18 A 65-year-old nonsmoker with HCV but no HBV, portal vein invasion, one liver lesion, no lymph nodes, and BCLC had 6 mutations (2 germline and 4 somatic). Somatic mutations have no data for somatic mutations.

Case 19 is a 67-year-old guy who has not smoked in 13 years, is positive for HCV but negative for HBV, with multiple liver lesions, no metastasis or lymph node, and is B child-Pugh. It involves a single PIK3CA mutation (W328G), previously characterized as a unique non-synonymous detrimental mutation.

Case 20 is a 53-year-old nonsmoker with negative HCV and HBV testing. No metastases or lymph nodes and 16 mutations in child-Pugh C with portal vein invasion (9 non-Synonymous,3 Synonymous,2 Germline, 2 Not Available). As indicated in Table (3), there are 2/9 harmful non-synonymous mutations (R683W and R683M) that may contribute to HCC development. Case 21 is a 67-year-old man with positive HCV, negative HBV, three liver lesions, lung metastases, no lymph nodes, and child-Pugh C.

One of the study's flaws is the small sample size. As a result, future cohort studies should be bigger. As a result, we urge that this study be performed with a larger cohort in the future. NGS was utilized to find multiple novel genetic variations in HCC, as well as well-known and rejected alterations. These findings propose a new way of looking at HCC's origin and development. To completely understand PIK3CA genetic changes and their influence on HCC development, bigger cohorts of patients would be necessary.

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اكتشاف الطفرات الجسدية للجين فوسفاتيدي لينوسيتول-4،5-ثنائي الفوسفات 3-كيناز بواسطة تسلسل الجيل التالي في سرطان الكبد لدى المصريين

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المستخلص

سرطان الخلايا الكبدية (HCC) هو ثالث أكبرسرطان خبيث يتسبب في الوفيات على مستوى العالم . يعني ارتفاع تكرار حدوثه أنه حتى في الوقت الذي يصبح فيه السرطان أكثر قابلية للعلاج في مراحله المبكرة، فإن الحالات المتقدمة يكون تشخيصها سيئًا. لعلاج سرطان الكبد بشكل صحيح، من الضروري ان نفهم عمليته المسببة للأمراض والتشوهات الجينية المرتبطة بها . تم استخدام تسلسل الجيل التالي (NGS) لاكتشاف الطفرات الجسدية لجين فوسفاتيدي لينوسيتول -4،5-ثنائي الفوسفات 3-كيناز(PIK3CA) في مرضى سرطان الكبد في مصر. في هذه الدراسة، تم استخدام لوحة NGS الفريدة (AmpliSeq) التي تحتوي على جين PIK3CA لفحص 21 عينة خزعة سائلة من مرضى سرطان الكبد . أشارت النتائج إلى أنه تم التعرف على أكثرمن 40 اختلافًا في النوكليوتيدات المفردة (SNV) في جين PIK3CA بنسبة حدوث 85.7 ٪. تم تفريق التغييرات الى ضارة ومتقبلة وغير محددة، حيث كانت غلبة التغييرات هي متغيرات الخطأ (68٪) والمرادفة (25٪) و (7٪) للتوقف المكتسب. استنتج من هذا البحث أن اكتشاف العديد من الطفرات الجسدية لـPIK3CA يمكن أن يساعد في عمل مسببات سرطان الكبد.