GENE SEQUENCING OF *EGFR* IN HEPATOCELLULAR CARCINOMA PATIENTS

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Hepatocellular carcinoma HCC is the fourth most prevalent cause of cancer-related mortality globally. Approximately 80-90% of HCC cases are associated with cirrhosis, which can be attributed to a chronic infection of the hepatitis B or hepatitis C virus (HCV). Many individuals with HCC are not candidates for potentially curative therapy such as surgical resection and transplantation due to their advanced stage of the disease Russo *et al*. (2022). In Egypt, it is the fourth most common cancer. Multiple examinations conducted in hospitals have shown an increase in the incidence of HCC Rashed *et al*. (2020).

The *EGFR* system regulates cell proliferation, survival, and migration. Its aberrant activity has been associated with the onset and progression of a variety of malignancies, including HCC. Overexpression of *EGFR* and its ligands has been associated with aggressive liver cancer with a low survival rate Berasain *et al*. (2011). *EGFR* overexpression is common in HCC, suggesting that it may play an important role in HCC etiology and treatment. Furthermore, *EGFR*

activation has been proposed as a potential predictor of primary resistance in HCC cells Guardiola *et al*. (2019). The objective of the present investigation was to examine the relationship between *EGFR* and the progression of HCC in HCC patients through the utilization of NGS technology.

MATERIALS AND METHODS

a. Study design and subjects

This study was conducted on a group of twenty-one patients who were suffering from HCC and were referred to the National Liver Institute Hospital at Menoufia University, Egypt. The study was approved and took place between January and November 2020. The patients, consisting of 18 males and 3 females with an average age of 62, had human HCC and were attending the oncology clinic at the National Liver Institute. The study only included patients suffering from HCC, and those with other types of cancers were excluded. The patient's genomes were analyzed against three healthy individuals who did not have any tumors. Each patient had a comprehensive evaluation, which included a clinical examination, tumor staging, thorough laboratory testing (such as coagulation profile, liver enzymes, renal function tests, and a complete blood count), and a chest X-ray. Menoufia University's Ethics Committee, namely the National Liver Institute, approved the project.

b. Sample collection and cell-free DNA extraction

Full blood samples were used for genomic DNA extraction after collecting 1-3 mL of peripheral blood in EDTA-containing tubes. A temperature of -80ºC was subsequently used to store the plasma. The QIAamp® DSP Virus spin kit Version 1 (QIAGEN, Hilden, Germany) was used to treat the plasma to extract DNA from circulating cells.

c. Next-generation sequencing

Genomic DNA was extracted using the Gene JET Genomic DNA Purification Kit (Thermoscientific, Cat#K0721). For library preparation, a total of 10 nanograms (ng) of DNA was amplified using the Ion AmpliSeqTM HiFi Master Mix and the Ion AmpliSeqTM Cancer Hotspot Panel (version 2; Thermo Fisher Scientific,Inc.).

The ion library TaqMan® Quantitation Kit (Thermo Fisher Scientific, Inc.) was used for qPCR quantification of the library according to the manufacturer's instructions. The templates were prepared and amplified using the Ion OneTouchTM2 technology. Thermo Fisher Scientific, Inc.'s Ionosphere quality control kit was used to ensure that 10% to 30% of the manufactured ISPs were template positive. Following enrichment, the template ISPs were transferred to Ion 316TM chips. The IonPGMTM Sequencing Hi-Q view kit v2 and PGMTM (Life Technologies) were

used for sequencing, following the manufacturer's instructions.

d Bioinformatics data analysis

The analysis of normal and malignant samples was conducted using the ion amplifier custom panel approach. The data was compared to Human Genome Version 19 (hg19) using Thermo Fisher's Ion reporter server 5.10, with the default plugin parameters employed. This comparison was conducted using Torrent Suite (version 3.6.2; Thermo Fisher Scientific, Inc.). The Coverage Analysis plugin (version 3.6; Thermo Fisher Scientific, Inc.) was employed in this study. The quality thresholds, average base coverage, allele frequency, and general uniformity were set at >20, >500x reads, >10%, and >80%, respectively. Mutations were discovered using the Variant Caller plugin (version 3.6; Thermo Fisher Scientific, Inc.). Subsequently, the validation of each mutation was conducted utilizing the Integrated Genome Viewer (IGV) provided by the Broad Institute (www.broadinstitute.org).

Statistical analysis

Using SPSS version 28, which was created in the Illinois city of Chicago, USA, the statistical assessment was carried out. Categorical variables were represented by frequencies and percentages, whereas continuous variables were represented by means, standard deviations, or medians (IQR). For continuous variables, we made use of the Mann-Whitney U test; for categorical variables, we used the Chi-square test to determine statistical significance. The threshold for statistical significance was set at $P<0.05$.

RESULTS AND DISCUSSION

There were 18 men (85.7% of the total) and 3 females (14.3%) in the research. Patients' ages were used to classify them into two groups: one for those 60 and above (61.9% of the total) and another for those 60 and younger (38.1%). The average age in group 1 was 62.19 ±8.85 years, while the median age was 63 years. We found that thirteen patients (61.9%) tested positive for bilharzia antibodies, nineteen patients (90.5%) tested positive for HCV, and one patient (4.75%) tested positive for HBV. Comorbidities are shown in (Table 1), with 7 cases (33.3%) having diabetes mellitus (DM) and 3 cases (14.3%) having hypertension (HTN). This agreed with Ikeda *et al*., (2018) who that found out of the 14 patients diagnosed with HCC, 85.7% were men with a median age of 62 years. Previous studies have linked the male predominance in HCC to increased exposure to risk factors, androgens (AR), and estrogens (ER) Zhang *et al*. (2020). As opposed to Tanaka *et al*. (2010), those who claimed that the higher prevalence of adenocarcinoma in females is reflected in the female predominance in HCC.

There was no statistically significant correlation between smoking and HCC. The association between HCC and tobacco exposure was further

supported by the finding of two out of the eight molecular markers that are linked to HCC. According to reports, smoking has several harmful consequences on the liver, such as liver carcinogens Li *et al*. (2019).

Based on the findings of this investigation, 61.9% of the 21 patients with HCC tested positive for bilharzia antibodies. These findings align with the research conducted by Ramadan *et al*. who found that Schistosoma antibodies of HCC Egyptian patients were (67.7%) of all 220 HCC patients Ramadan *et al*. (2021). The risk of developing primary liver cancer was shown to be up to 50% higher in people who smoked compared to those who did not smoke. It was also determined that 64 percent of Egyptians diagnosed with HCC smoke. In Egypt, excessive smoking is a major risk factor for non-B or non-C HCC, according to Abou El Azm *et al*. (2014). According to another research, smoking is a major factor in the development of HCC in Egypt Brozzetti *et al*. (2021). A second Egyptian study found that people who smoked 20 cigarettes daily for over 29 years had a higher chance of developing HCC Moustafa *et al*. (2009).

According to this study, anti-HCV antibodies were present in 90.5% of all 21 patients, but only 4.75% of total cases had HBV. It was demonstrated that HCV was the predominant cause of HCC in Egypt, and it continues to be the main factor in the development of HCC in Europe, North America, and Japan Brozzetti *et al*. (2021).

The results of this study agreed with those of an earlier one that indicated that 33.5 percent had a history of heavy alcohol consumption, 24.3 percent had viral hepatitis, and 33.5 percent had both diagnoses. Among the participants, 29.9% had diabetes, 37.9% had hypertension, and 35.9% smoked cigarettes Raffetti *et al*. (2015). Among the cohort of 21 patients, there is 19.0% had a familial predisposition to liver cancer. There is no significant correlation between a familial predisposition and HCC. Nevertheless, Caruso *et al*. have observed a significant correlation between a familial background of liver cancer and a heightened susceptibility to the development of HCC characterized by aggressive characteristics Caruso *et al*. (2017).

According to this study, DM incidence was in only 33.3% of all HCC cases with the same line of the baseline clinical characteristics of the previous study of the whole study population that indicated that DM incidence was present in (24.1%) among all HCC patients Ramadan *et al*. (2021). Second research found that having type 2 diabetes doubles or even triples the probability of having HC Ziada *et al*. (2016).

The rise in HCC identification in Egypt may be attributed to the implementation of a comprehensive screening program aimed at finding and treating HCV. Several people were diagnosed and treated for HCC as a result of this initiative. Other researchers conducted a study that revealed that 75%

of hepatocellular carcinoma (HCC) cases originated from rural regions in Egypt. Additionally, 45.7% of the affected individuals fell within the age range of 51-60 years Zhao *et al*. (2020).

This study found that ascites were identified in (19.0%) of patients (3 minimal and 1 moderate) of all 21 patients at the time of diagnosis. Three (14.3%) HCC patients with (PVI) were significantly correlated with HCC (P = 0.01). The most common metastatic sites were the lungs (14.3%) and the lymph nodes (14.3%). A previous study reported that brain metastasis was (2%), peritoneum (11%), adrenal glands (11%), bone (28%), local lymph nodes (53%), and lung (55%) which were the most frequent extrahepatic HCC metastatic locations to their frequencies Zhao *et al*. (2020).

Extrahepatic metastasis is a sign of advanced HCC, according to clinical standards. The classic Child-Pugh rating system has been the most popular way to evaluate liver function and determine the effectiveness of treatments for many years Zhao *et al*. (2020). Various staging systems, including the BCLC staging system, have been proposed in recent years. The Child-Pugh score, tumor burden, and patient performance status are only a few factors the BCLC staging system considers Hsu *et al*. (2013).

According to this current study, 81% of the cases had tumors measuring more than 5cm in diameter based on CT scans of the population. Among these cases, 57.1% had multiple lesions lesion, and 42.9% had a single lesion. According to the BCLC staging, stages A and C had a higher prevalence rate of 33.35% apiece. The results of the Child-Pugh score indicate that Child's A accounted for 76.2% of the total, followed by Child's B at 14.3% and Child's C at 9.5% (Table 2). The aforementioned results align with the data reported by Hassan-Kadle *et al*. (2022)., wherein it was seen that 73.6% of the patients fell into the Child-Pugh classification, with 17.2% classified as Child's A and 9.0% classified as Child's C Furthermore, the aforementioned findings were consistent with the research which reported that 64.3% of patients exhibited Child-Pugh B or C cirrhosisIkeda *et al*. (2018). According to another research, 162 patients (73.6%) had portal veins, with 104 patients (47.3%) having multiple lesions in the right lobe. Additionally, 180 patients (81.8%) had cirrhosis, 104 patients (47.3%) had BCLC stage D, and 105 patients (47.7%) were classified as child B %) Ramadan *et al*. (2021).

Based on the demographic and clinical data at hand, it is evident that the *EGFR*-mutated group had a notably greater representation of male participants, non-smokers, in contrast to the Wild-Type group, as well as those whose tumors were either mildly or fairly differentiated. Based on our research, it appears that the higher frequency of *EGFR* mutation in males may be linked to a higher incidence of HCC. Our findings indicate that the higher prevalence of *EGFR* mutations in

males is indicative of a greater occurrence of HCC. The correlation between patients and *EGFR* mutations was significantly more likely to be male (84.2%). *EGFR* gene mutations were also significantly more likely to be non-smoker (73.7%) and without family history (78.9%). Out of all muted patients significantly more likely to have HCV (89.5%), 78.9% without ascites, 15.8% had positive PVI, 5.3% had metastasis in lung and lymph node and 15.8% had HTN. The large tumor size (more than 5 cm) was significantly predominant (84.2%). There was a negative correlation between *EGFR* gene mutation in HCC cases and clinical characteristics and clinic-pathological features. as shown in (Tables 3&4).

According to a study conducted by Lin *et al.* (2020), there were more women and people without smoking in the *EGFR* mutant group, and there were more people with tumors that were well- or moderately differentiated compared to the wild-type group. These findings are in direct opposition to prior research.

The study revealed that there was no significant correlation between *EGFR* mutation status and demographic and clinical features, including age, degree of differentiation, clinical stages, tumor size, viral infection, and other pathological differentiation ($P > 0.05$). The research that demonstrated that there is no correlation between age and pathogenic differentiation with *EGFR* mutations Zhou *et al.* (2019). On the other hand, another study determined that the overall number of poor and moderate differentiation was lower in *EGFR* mutation-harboring SqCLC patients compared to wild-type patients Zhang and Junling (2016).

All patients underwent *EGFR* gene sequencing, and mutations were identified in 19/21 (90.5%) of the samples. All detected mutations were 55 variants (Table 5). There were 39/55 (70.9%) single nucleotide variants (SNVs), 3/55 (5.5%) multi-nucleotide variants (MNVs), 8/55 (14.5%), copy number variants (CNVs) and 5/55 (9.1%) insertions/deletion variants (INDELs). The Ensembl Variant Effect Predictor (VEP) was utilized to further interpret and filter genomic variants. It predicts the molecular consequences of variants using the Ensembl/GENCODE or RefSeq gene sets. Out of the SNVs and INDELs, 26 out of 44 (59.1%) were found to be somatic mutations, while 18 out of 44 (40.9%) were germline mutations. There were also both novel and existing mutations present, with 26 out of 44 (59.1%) being novel and 18 out of 44 (40.9%) being existing mutations. The Variant effect showed that there were 14 (31.8%) missense variants, 6 (13.6%) synonymous variants, 18 (40.9%) coding transcript intronic variants, 1 (2.3%) stop-gained variants, 4 (9.1%) splice region variants and 1 (2.3%) splice donor variants. Predicted ACMG Outcome by VEP showed that 3 (6.8%) were Likely pathogenic, 13 (29.6%) Uncertain significance, 2 (4.5%) Benign, 26 (59.1%) Likely benign (figs. 1&2&3).

A previous study aimed to assess the frequency of concurrent genetic changes in a cohort of 54 individuals diagnosed with advanced lung cancer. To achieve this, a series of gene assays were conducted, encompassing a range of somatic genetic alterations Deng *et al*. (2019). Specifically, the study focused on 24 patients with *EGFR* mutations and 30 patients with *EGFR* mutations who were in the advanced stage of lung cancer (stage IIIB or IV). NGS was used to test copy number variants (CNV), inframe_indel, fusions, frameshift, missense, splicing, and stop acquired in 422 clinically important cancer genes. The findings of this current study are consistent with our research outcomes. Among the mutant type of *EGFR*, there were 4 out of 24 CNVs, 8 out of 24 inframe indel mutations, and 16 out of 24 missense mutations. In contrast, the wild-type *EGFR* had less than 2 out of 30 CNVs and no other differences were observed Deng *et al*. (2019).

On the other hand, there was study that evaluated 14 patients with advanced HCC. The calculation was performed to determine the proportion of mutant alleles concerning wild-type alleles. Every individual in the study exhibited at least one somatic mutation, with a median of three mutations per patient (ranging from 1 to 8). The mutant alleles had a median percentage of 0.29%, with a range of 0.1% to 37.77%. A total of 14% of mutations were detected in the *EGFR* gene. Furthermore, a comprehensive analysis was conducted on the complete exons of 29 genes, as well as critical exons specifically identified as having somatic variants in the COSMIC dataset. The purpose of this investigation was to detect and record SNVs, amplifications of 16 genes' copy numbers, fusions of ALK/RET/ROS1/NTRK1, and *EGFR* insertion/deletion mutations Ikeda *et al*. (2018).

An investigation was carried out on the *EGFR* gene in 100 patients with HCC and 102 patients with nasopharyngeal carcinoma, with a specific focus on exons 18-21. A silent exonic mutation in exon 20, 2361G > A (Q787Q), was identified in 32% of samples from HCC and 41% of samples from nasopharyngeal cancer, according to the study. In exon 20, a silent exonic mutation, specifically 2457G > A (V819V), was identified and was observed in 3% of the samples of nasopharyngeal cancer. There were no further mutations detected in exons 18 to 21 in the samples of hepatocellular and nasopharyngeal cancer. Eight intronic mutations were identified Lee *et al*., (2006).

SUMMARY

Limitations: found no evidence of portal vein invasion but did associate *EGFR* mutation with hypertension. Larger tumors tend to have more *EGFR* mutations. Further investigations, such as whole exome sequencing, are required to comprehensively elucidate the genetic modifications in HCC NGS facilitated the identification of numerous distinct gene variants in HCC, encompassing both validated and invalidated mutations. The

understanding of the origin and course of hepatocellular carcinoma (HCC) is enhanced by these results, which provide novel perspectives. To completely understand the impact of *EGFR* genetic change on the development of HCC, it is necessary to have larger patient cohorts.

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Table (1): HCC patient characteristics and risk factors.

Variables		HCC (n = 21) No. (%)	OR (95% CI)	P-value
Sex	**Male**	18 (85.7%)	-	-
	Female	3 (14.3%)	-	-
Age (years)		62.19 ±8.85	-	-
Age groups (years)	**<60** **>60**	8 (38.1%) 13 (61.9%)	-	-
Risk factors				
Smoking	**Yes** **Ex. Smoker**	2 (9.5%) 5 (23.8%)	NA	0.213
Bilharzia	**Yes**	13 (61.9%)	1.625 (0.558- 4.730)	0.373
Hepatic encephalopathy	**Yes**	0 (0.0%)	0.023 (0.001- 0.409)	0.01*
Family history	**Yes**	4 (19.0%)	0.235 (0.068- 0.818)	0.023*
Viral infection	**HCV** **HBV** **NBNC**	19 (90.5%) 1 (4.75%) 1 (4.75%)	9.50 (1.96- 46.01) 0.050 (0.006- 0.407) 0.050 (0.006- 0.407)	0.005*
Comorbidities	**DM** **HTN**	7 (33.3%) 3 (14.3%)	0.50 (0.168- 1.488) 0.167 (0.043- 0.652)	0.213 0.01*

Data are presented as frequency (%) or mean ± SD. HCC: Hepatocellular carcinoma, HCV: hepatitis C virus, HBV: hepatitis B virus, DM: diabetes mellitus. HTN: hypertension. *Significant. P value <0.05, CI: confidence interval, OR: odds ratio.

Table (2): Clinicopathological features of HCC patients.

Variables		HCC (n = 21) No. (%)	OR (95% CI)	P- value
Ascites	**No** **Minimal** **Moderate**	17 (81.0%) 3 (14.3%) 1 (4.7%)	NA	0.023*
Portal Vein Invasion	**Negative** **Positive**	18 (85.7%) 3 (14.3%)	6.00 (1.53- 23.47)	0.01*
LN Metastasis	**Negative** **Positive**	18 (85.7%) 3 (14.3%)	6.00 (1.53- 23.47)	0.01*
Lung Metastasis	**Negative** **Positive**	18 (85.7%) 3 (14.3%)	6.00 (1.53- 23.47)	0.01*
Child PUGH class	**A** **B** **C**	16 (76.2%) 3 (14.3%) 2 (9.5%)	NA	0.05*
CT radiological findings				
Tumor number	**Single** **Multiple**	9 (42.9%) 12 (57.1%)	-	-
Tumor Size	**Small (<3 cm)** **Medium (3 - 5 cm)** **Large (>5 cm)**	3 (14.3%) 1 (4.7%) 17 (81.0%)	-	-
BCLC	**A** **B** **C** **D**	7 (33.35%) 5 (23.8%) 7 (33.35%) 2 (9.5%)	-	-

Data are presented as frequency (%) or mean ± SD. BCLC: Barcelona clinic liver cancer. *Significant. P value <0.05.

Table (3): *EGFR* mutation in HCC cases according to demographic and clinical characteristics.

Variables		Mutant type (n=19)		Wild type (n=2)		OR (95% CI)	*P*-value
		No.	%	No.	%		
Sex	**Male**	16	84.2%	2	100%	0.842 (0.106-6.672)	0.871
	Female	3	15.8%	0	0%		
Smoking	**Yes**	2	10.5%	0	0.0%	NA	0.408
	No	14	73.7%	0	0.0%		
	Ex. smoker	3	15.8%	2	100%		
Bilharzia	**Yes**	13	68.4%	0	0.0%	3.462 (0.154- 77.98)	0.435
	No	6	31.6%	2	100%		
Hepatic encephalopathy	**Yes**	0	0.0%	0	0%	1.00 (0.262-3.815)	1.00
	No	19	100.0%	2	100%		
Family history	**Yes**	4	21.1%	0	0.0%	1.154 (0.047-28.44)	0.930
	No	15	78.9%	2	100%		
Viral infection	**HCV**	17	89.5%	2	100%	0.895 (0.113-7.065)	0.916
	HBV	1	5.25%	0	0%	0.385 (0.012- 12.249)	0.588
	NBNC	1	5.25%	0	0.0%	0.385 (0.012- 12.249)	0.588
Comorbidities	**DM**	6	31.6%	1	50%	0.632 (0.048-8.252)	0.726
	HTN	3	15.8%	0	0.0%	0.897 (0.035-22.975)	0.948

Data are presented as frequency (%) or mean ± SD. HCC: Hepatocellular carcinoma, HCV: hepatitis C virus, HBV: hepatitis B virus, DM: diabetes mellitus. HTN: hypertension. *Significant. P value <0.05, CI: confidence interval, OR: odds ratio.

Table (4): *EGFR* mutation in HCC cases according to clinicopathological characteristics.

Variables	Mutant type (n = 19)		Wild type (n=2)		*P*- value
	No.	%	No.	%	
Ascites					
No	15	78.9%	2	100%	0.823
Minimal	3	15.8%	0	0.0%	
Moderate	1	5.3%	0	0.0%	
Portal Vein Invasion					
Negative	16	84.2%	2	100%	0.871
Positive	3	15.8%	0	0.0%	
LN Metastasis					
Negative	18	94.7%	0	0.0%	0.325
Positive	1	5.3%	1	100%	
Lung Metastasis					
Negative	18	94.7%	0	0.0%	0.325
Positive	1	5.3%	1	100%	
Child PUGH class					
A	14	73.7%	2	100%	0.773
B	3	15.8%	0	0.0%	
C	2	10.5%	0	0.0%	

Table (4):Cont’					
CT radiological findings					
Tumor number					
Single	9	47.4%	0	0.0%	0.578
Multiple	10	52.6%	2	100%	
Tumor Size					
Small (<3 cm)	2	10.5%	1	50%	0.682
Medium (3 - 5 cm)	1	5.3%	0	0.0%	
Large (>5 cm)	16	84.2%	1	50%	
BCLC					
A	7	36.9%	0	0.0%	0.684
B	5	26.3%	0	0.0%	
C	5	26.3%	2	100%	
D	2	10.5%	0	0.0%	

Data are presented as frequency (%) or mean ± SD. PVI: portal vein invasion. BCLC: Barcelona clinic liver cancer. *Significant. P value <0.05.

Table (5): Summary of *EGFR* gene variation in HCC detected by targeted sequencing.

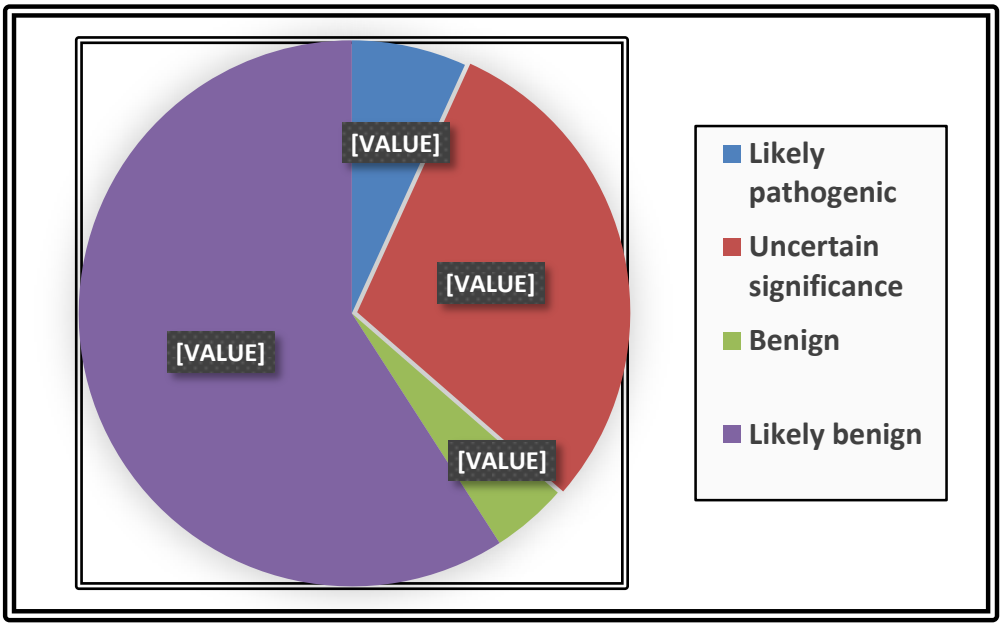
Locus	Types	Variant frequency	Genes	Amino acid change	Coding
chr7:55211044	**CNV**	0.28	*EGFR*-AS1, MET, *EGFR*	0	0
chr7:55211044	**CNV**	0.04	*EGFR-AS1, EGFR*	0	0
chr7:55211044	**CNV**	0.12	*EGFR*	0	0
chr7:55211044	**CNV**	0.08	*EGFR*	0	0
chr7:55211044	**CNV**	0.12	*EGFR*	0	0
chr7:55211097	**SNV**	0.04	*EGFR*	p. Glu114Ter	c.340G>T
chr7:55221798	**SNV**	0.04	*EGFR*	p. Pro281Leu	c.842C>T
chr7:55221824	**SNV**	0.04	*EGFR*	p. Thr290Ala	c.868A>G
chr7:55221871	**SNV**	0.12	*EGFR*	#N/A	#N/A
chr7:55221872	**SNV**	0.16	*EGFR*	p.?	c.889+27A>G
chr7:55221874	**INDEL**	0.04	*EGFR*	p.?	c.889+32delT
chr7:55221877	**SNV**	0.04	*EGFR*	p.?	c.889+32T>G
chr7:55221881	**SNV**	0.08	*EGFR*	p.?	c.889+36G>T
chr7:55221883	**MNV, INDEL**	0.12	*EGFR*	p.?	c.889+38_889+39insC ...(2)
chr7:55221884	**SNV**	0.04	*EGFR*	p.?	c.889+39T>C
chr7:55221886	**INDEL**	0.04	*EGFR*	p.?	c.889+41_889+42insAG
chr7:55221887	**SNV**	0.04	*EGFR*	p.?	c.889+42T>G

Table (5):Cont'

chr7:55221891	**SNV**	0.04	*EGFR*	p.?	c.889+46C>G
chr7:55221893	**SNV**	0.04	*EGFR*	p.?	c.889+48C>G
chr7:55221894	**SNV**	0.04	*EGFR*	p.?	c.889+49G>T
chr7:55232962	**CNV**	0.04	*EGFR*-AS1, MET, *EGFR*	0	0
chr7:55232962	**CNV**	0.04	*EGFR*-AS1, *EGFR*	0	0
chr7:55233038	**SNV**	0.04	*EGFR*	#N/A	#N/A
chr7:55233052	**SNV**	0.04	*EGFR*	p. Gly601Ala	c.1802G>C
chr7:55241635	**CNV**	0.04	*EGFR-AS1, EGFR*	0	0
chr7:55241637	**SNV**	0.08	*EGFR*	p. Ser695Arg	c.2085T>G
chr7:55241674	**SNV**	0.04	*EGFR*	p. Lys708Glu	c.2122A>G
chr7:55241725	**SNV**	0.16	*EGFR*	p. Thr725Pro	c.2173A>C
chr7:55241728	**SNV**	0.04	*EGFR*	p. Val726Leu	c.2176G>C
chr7:55242412	**SNV**	0.04	*EGFR*	#N/A	#N/A
chr7:55242453	**SNV**	0.08	*EGFR*	p. Pro741=	c.2223C>A
chr7:55248970	**SNV**	0.04	*EGFR*	p.?	c.2284-16C>T ... (2)
chr7:55248973	**INDEL**	0.04	*EGFR*	p.?	c.2284-13_2284-12insATTTATGTGGA ... (2)
chr7:55248978	**SNV**	0.04	*EGFR*	p.?	c.2284-8C>G

Table (5):Cont’					
chr7:55249070	**INDEL**	0.04	*EGFR*	p. Thr790SerfsTer36	c.2369delC
chr7:55249074	**SNV**	0.16	*EGFR*	#N/A	#N/A
chr7:55249078	**SNV**	0.08	*EGFR*	#N/A	#N/A
chr7:55249189	**SNV**	0.12	*EGFR*	#N/A	#N/A
chr7:55249193	**SNV**	0.08	*EGFR*	#N/A	#N/A
chr7:55249194	**SNV**	0.08	*EGFR*	p.?	c.2469+23G>A
chr7:55249198	**SNV**	0.12	*EGFR*	p.?	c.2469+27G>A
chr7:55249210	**SNV**	0.04	*EGFR*	p.?	c.2469+39A>T
chr7:55259541	**SNV**	0.12	*EGFR*	#N/A	#N/A
chr7:55259542	**SNV**	0.12	*EGFR*	#N/A	#N/A
chr7:55259546	**SNV**	0.04	*EGFR*	#N/A	#N/A
chr7:55259548	**SNV**	0.04	*EGFR*	#N/A	#N/A
chr7:55259555	**SNV**	0.04	*EGFR*	p. Ala871=	c.2613A>T
chr7:55259558	**SNV**	0.08	*EGFR*	p. Glu872Asp	c.2616A>T
chr7:55259561	**SNV**	0.04	*EGFR*	p. Gly873=	c.2619A>G
chr7:55259568	**SNV**	0.04	*EGFR*	p.?	c.2625+1G>C
chr7:55259570	**MNV**	0.04	*EGFR*	p.?	c.2625+3_2625+5delinsGCT
chr7:55259574	**SNV**	0.04	*EGFR*	p.?	c.2625+7A>T
chr7:55259577	**MNV, SNV**	0.04	*EGFR*	p.? p.?	c.2625+10_2625+14delinsACCTA, c.2625+12G>T

SNV: Single nucleotide variation, CNV: copy number variation, MNV: multi-nucleotide variant, N/A: not applicable, **INDEL:** insertions/deletion variants.



FFig. (1): Percentage of Predicted Outcome by VEP.

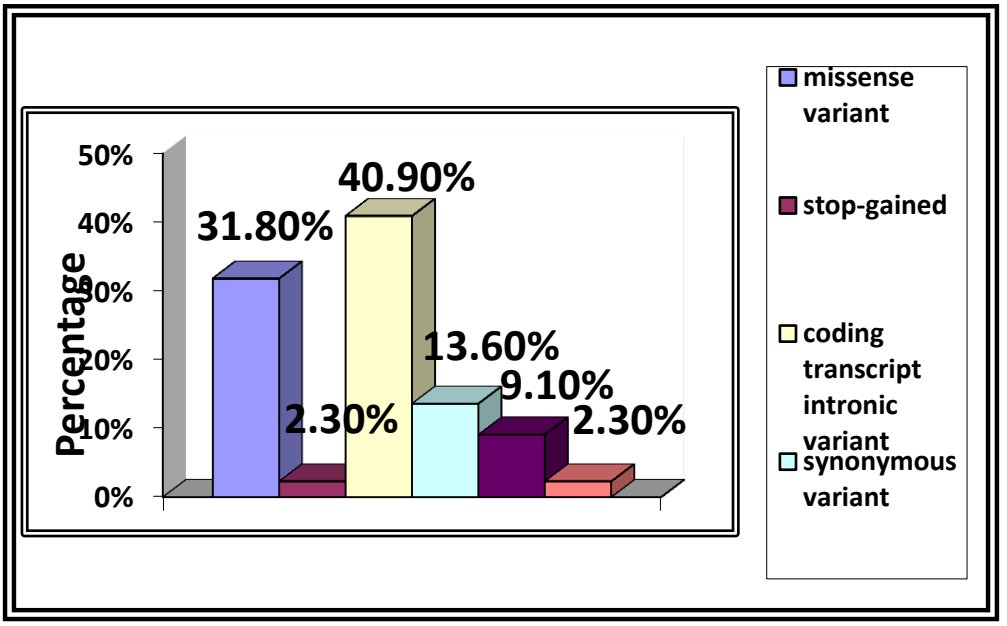


Fig. (2): Variant effect of SNVs

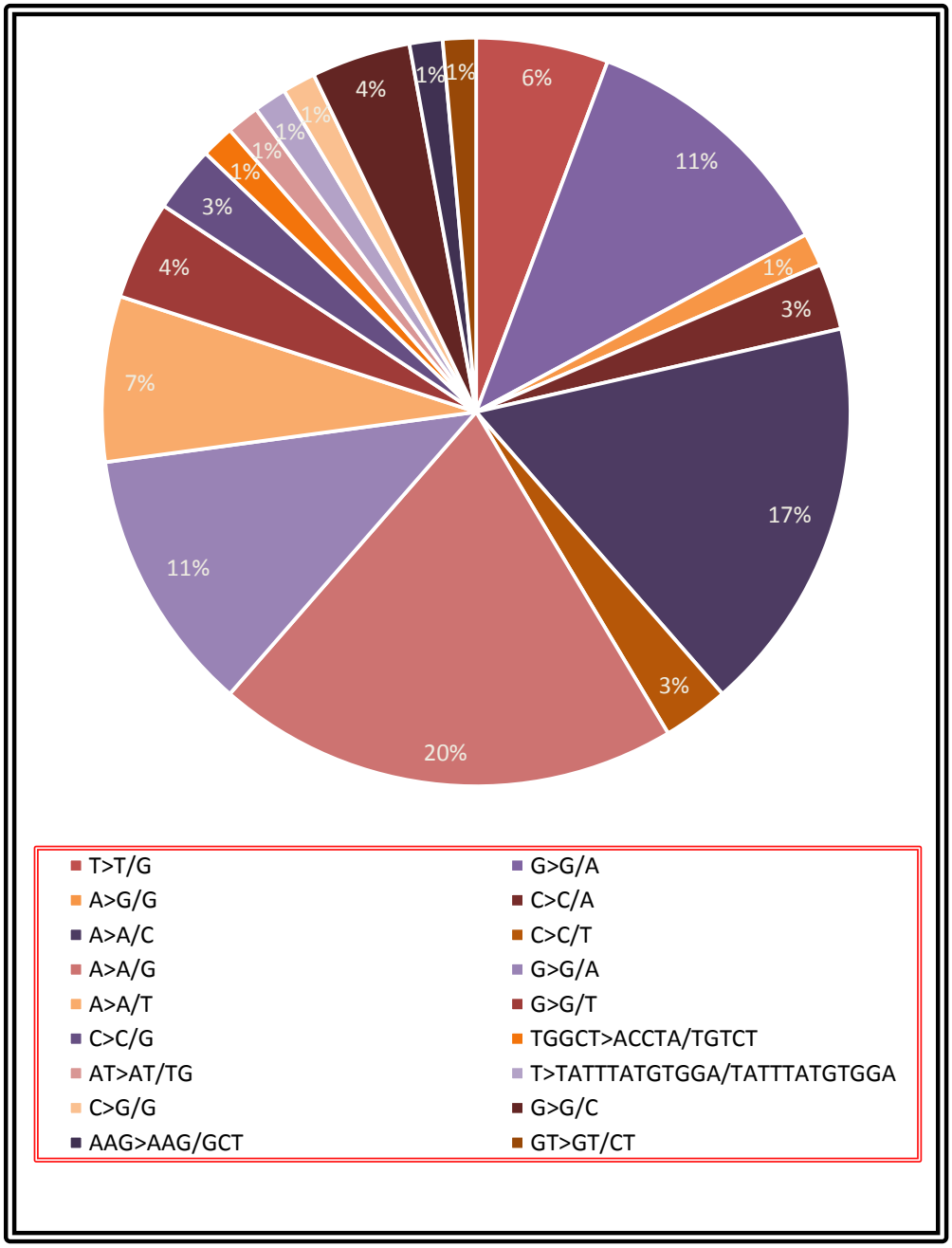


Fig. (3): **Summary of EGFR mutations among HCC patients.**