GENETIC PROFILING OF FGFR2 GENE IN EGYPTIAN HEPATOCELLULAR CARCINOMA PATIENTS USING NEXT-GENERATION SEQUENCING

WAEL SOLIMAN [1], MOHAMED BIOMY [1], GHADA M. NASR [1], MANAL O. ELHAMSHARY [1], M. I. NASR [2], ABDELREAHMAN ABDELHALIM [1].

1. ***Department of Molecular Diagnostic and Therapeutic, Genetic Engineering & Biotechnology Research Institute, University of Sadat city, Egypt***
2. ***Department of Molecular Biology, Genetic Engineering & Biotechnology Research Institute, University of Sadat city, Egypt***

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Hepatocellular carcinoma (HCC) is the 2nd most common cause of cancer-related death worldwide. Globally, the prevalence of HCC is increasing, and in Egypt, during the past ten years, the incidence has nearly doubled (Ali *et al*., 2023). Aflatoxin exposure, excess body weight, type 2 diabetes, alcohol-related liver disease (ALD), hepatitis C (HCV), HBV, and cirrhosis are risk factors for HCC, which is developed in the majority of patients. HCV is the leading cause of HCC in Egypt (Abboud *et al*., 2024 and Kinsey and Lee, 2024). Alpha-fetoprotein (AFP) biomarker testing and abdominal ultrasonography are standard screening methods for HCC. Because it is widely available and reasonably priced, abdominal ultrasonography is the favored imaging modality over magnetic resonance imaging (MRI) and computed tomography (CT) (Parra *et al*., 2023).

In HCC, heterogeneity is a well-documented phenomenon that leads to cellular, molecular, functional, and lineage variety. It is assumed to be caused by environmental variables and patients' differing genetic diversity (Safri *et al*., 2024). The efficient treatment of HCC is further complicated by the incomplete understanding of the molecular pathways underlying tumour growth and progression. Furthermore, a significant knowledge vacuum about HCC is shown by the absence of indicators unique to tumour type or disease stage (Panneerselvam *et al*., 2023). With the use of next-generation sequencing (NGS) technologies, we have fully characterized the genes that are most often mutated in HCC. In HCC samples, pathogenic

mutations in many genes have been identified. Notably, NGS has been used to identify the most frequently altered genes associated with HCC (Valiante and Grammatico, 2023).

The fibroblast growth factor receptors (FGFRs), are a family of receptor tyrosine kinases (RTKs) that go by the name FGFR1–5. A transmembrane, intracellular tyrosine kinase domain, and FGF ligand-binding extracellular domain are present in all FGFRs with the exception of FGFR5 (Shan *et al*., 2024). In order for cells to proliferate, migrate, survive, develop into embryos, maintain homeostasis, heal damaged tissue, and maintain apoptosis, FGFRs must be activated by FGFs. According to Mahapatra *et al.* (2023), FGFR gene amplifications, mutations, and fusions establish dysregulation in the FGFR signalling pathways, which in turn causes oncogenesis, tumour growth, and angiogenesis as well as resistance to anticancer treatment. Surprisingly FGFR2 polymorphism was found in HCV-positive HCC patients in a relatively recent investigation, indicating a potential link between FGFR2 polymorphism and HCV-induced HCC (Al-Khaykanee *et al*., 2021; Khan et al., 2022).

Thus, the main objective of this study was to use NGS data to find new FGFR2 mutations that are not known to cause HCC in Egyptian patients and to correlate those variants with clinically relevant characteristics.

MATERIALS AND METHODS

Study design and patients

Twenty-one patients with hepatocellular carcinoma (aged 48–80) who attended the National Liver Institute at Menoufiya University's HCC clinic were included in the current study. A patient's age, sex, medical history (including hepatitis, diabetes mellitus, and Hypertension), smoking habits, and the presence of cirrhosis (ascertained by physical examination, radiography using CT and ultrasound imaging, and guided liver biopsy in patients), clinical symptoms, lab results, Child-Pugh class, size, and number of primary tumours are all taken into consideration. The Menoufia University ethics committee approved the study (NLI IRB process 00232/2020, December 2020). The trial did not take on other cancer patients.

Sample collection and Extraction of free cell DNA

Each HCC patient was provided 5 ml of peripheral EDTA blood in a vacutainer tube. The plasma was separated using two centrifugation stages-2,000 x g at 4 °C for 10 minutes and 16,000 xg at 4 °C for 10 minutes within an hour. Samples of plasma were immediately separated and kept at -80°C for up to nine months. The Qiagen "QIAamp Circulating Nucleic Acid Kit" was utilized to extract and purify cell-free

DNA. The Qubit™ 3.0 Fluorometer (Life Technologies, Thermo Fisher Scientific, Inc.) was used to quantify and purify the isolated cfDNA.

Next-generation sequencing

Ten nanograms of collected cfDNA were used to create the sequencing library, and the FGFR2 gene was amplified using multiplex PCR utilizing the Ion AmpliSeq™ HiFi and Custom AmpliSeq NGS Panel. After the amplicons were fragmented, they were ligated to an adaptor and amplified using a thermocycler following the manufacturer's instructions. The library was purified using the DynaMag™-96 Side Magnet and the Agencourt™ AMPure™ XP Reagent to eliminate unbounded adapters. The library was measured using the StepOne Real-Time PCR and the Ion Library TaqMan® Quantitation Kit. Using the Ion One TouchTM 2 Instrument and the Ion PGMTM Hi-Q™ View OT2 Kit – 200, the library pieces were applied to Ionsphere particles (ISPs). The quality control of the library was evaluated using the Qubit™ 3.0 Fluorometer and the Ion Sphere™ Quality Control Kit (Thermo Fisher Scientific, Inc.). The Ion 316™ chip underwent meticulous loading and enrichment of the template ISPs. Following the manufacturer's instructions, the Ion 316™ chip was placed into the Ion Torrent PGM (Life Technologies, Thermo Fisher Scientific, Singapore) and sequenced using sequencing kits (Ion PGMTM Hi-Q™ View Sequencing 200 Kit v2-Thermo Fisher Scientific, Inc.).

Data and sequence variants analysis

The "cloud-based Ion reporter server version 5.10" on the ThermoFisher website received the created BAM files. The ion ampliseq cancer hotspot panel methodology was used to analyze the matched normal and tumour samples using the default plugin parameters. The following databases were used to find prior reports of sequence variants: NCBI (https://www.ncbi.nlm. nih.gov/), COSMIC (http://cancer. sanger.ac.uk/cosmic), and Ensembl (https://asia.ensembl.org/index.html).

Statistical analysis

The continuous variables' data were presented as mean ± Standard Deviation or median (IQR), and the categorical variables' data were presented as frequencies and percentages. Mann-Whitney U tests were used for continuous data, while the Chi-square test was used for categorical data to look at the association between the variables. $P<0.05$ was considered as the statistical significance cut-off point. The statistical analysis was conducted using SPSS version 28 (Chicago, IL, USA).

RESULTS AND DISCUSSION

Genetic and environmental factors contribute to HCC vulnerability, according to growing evidence. Liver cancer involves several molecular, cellular, and histological steps. Chronic liver inflammation may damage, kill, and regenerate hepatocytes, changing their

epigenetic and genetic makeup (Yang *et al*., 2023). This study sought to determine the risk factors for HCC and estimated that if FGFR2 gene alterations were associated with the development of HCC.

In the current study, males were predominant, representing 18 (85.7%) of the study population with a male-to-female ratio of 6 to 1. With a mean age of 62.19 ± 9.08 and a median age of 63 years, among them, 13 (61.9%) were <60 years old and 8 (38.1%) were ≥60 years old. These study finding were in the line with previous studies, Egypt's age at which HCC first manifested itself differed significantly from that of 11 other African nations, according to the Africa Liver Cancer Consortium. In Egypt, the average age was 58 years, while in other nations it was 46 years (Okeke *et al*., 2020). The male-to-female ratio varies depending on the location, from 2:1 to 7:1. In general, HCC in females is less progressed and smaller (Zhang *et al*., 2021). The increased proportion of men relative to women among the patients under study is consistent with findings from previous HCC investigations, including those documented by Shen *et al*. (2023). These results show a significant male predominant in liver cancer prevalence, particularly in those under 60 (Chen and Chang, 2023). According to Thokerunga *et al*., (2023), AFP is the most often used serum tumour marker for HCC in terms of diagnosis, response to therapy, and prognosis. The blood AFP mean level was 2304.08 ± 9245.31 in this study, while the median level was 44.2 ng/dL. In this study, Patients with HCC had blood levels of AFP that varied from 4.9 to 42443 ng/ml, with a mean of 2304.08 ± 9245.31ng/ml and a median of 44.2 ng/ml.

The complete patient demographics and clinicopathological characteristics was represented in Table (1). HCV was the main cause of HCC in this research and remains the main cause of HCC to this day (Shen *et al*., 2023). Egypt's HCC incidence has about doubled in the past ten years; the country's high HCV prevalence may be the cause of this rising incidence. According to our study, the prevalence rates of HCV antibody among HCC patients was (85.7%). This finding is consistent with Sayiner *et al*., (2019) estimate that 84% of HCC cases in Egypt had HCV as their cause. Just 4.75% of HCC patients in the current research tested HBs Ag positive, despite the fact that HBV infection is widely recognized as a major risk factor for hepatic cirrhosis and eventual HCC. This data was at odds with that of Fathy Barakat *et al*., (2021), who showed that the prevalence of HBV was around 34.04% among Egyptian patients with HCC. This change may be the result of a difference in sample size. In the current study, 61.9% of the 21 HCC patients had bilharzia antibodies. According to Ramadan *et al*. (2021), 67.7% of Egyptian HCC patients had bilharzia antibodies. This result is consistent with their findings. In the current study, 33.3% of the HCC patients reported having diabetes mellitus. This finding corroborated that type 2 diabetes

mellitus affected 39.7% of Egyptian HCC patients, according to Elkenawy *et al.* (2022). Furthermore, there is evidence associating primary hypertension with HCC mortality (Lopez-Lopez *et al*., 2020). Hypertension was detected in 14.3% of the HCC patients.

Ascites is primarily one of the main consequences and a sign of a deteriorating liver functional reserve, but it may also be a tumour growth marker. Ascites were seen in 19% of the HCC patients in our study. The findings of this study are consistent with those of Liao *et al.* (2023), who found that 22.5% of HCC patients had ascites at the time of diagnosis. According to Shehta *et al*., (2021), a number of studies have demonstrated the prevalence of portal vein invasion (PVI), which is probably underreported and occurs in 30% to 62% of patients with advanced HCC. PVI was discovered in 14.3% of the patients in this study sample. This is consistent with the findings of Al-Haimi *et al.* (2018), who found that 18.9% of patients had PVT at the time of HCC diagnosis. Finding extrahepatic metastases is necessary to choose the best course of treatment since they are a recognized independent predictor of poor survival (Sarma et al., 2021). Each of the study's metastatic sites, lymph nodes and lung metastases accounted for 14.3%. This conclusion aligns with studies by Deo *et al.* (2021) that found 22.2% of patients had regional lymph node metastases and Ganeshan *et al.* (2018) that found the lung to be the most prevalent site for metastases.

Regarding our scoring system, a common feature incorporated into most HCC prognostic models is tumour burden, defined as the total number and size of tumours (Kaewdech *et al*., 2023). In this study, 52.4% of the HCC patients had multiple lesions. In 76.2% of the study population, most had large tumours (> 3). This result was less than that of Ali *et al*., (2023), who reported that 87.1% of patients with focal lesions had three lesions or fewer, and 12.9% had more than three lesions. For many years, the Child-Pugh grading system was the most common technique for assessing liver function and determining how suitable treatments were working (Zhao *et al*., 2020). With HCC patients, Child-Pugh A was the most prevalent (76.2%), followed by Child-Pugh B in 14.3% and Child-Pugh C in 9.5%. This result is consistent with the findings of Elkenawy *et al.* (2022), who observed that child A patients had a higher prevalence of HCC than child B and C. The staging of HCC is crucial for prognostic assessment and selecting the most effective treatment strategy. Regarding prognostic prediction, the most widely used staging approach is the Barcelona Clinic of Liver Cancer (BCLC) staging system (Borde *et al*., 2022). Based on BCLC staging, stages A and C were shown to be more common (33.3% each) in this study. The results of this study support those of Que *et al.* (2020), who reported that stage C is the

most prevalent stage of BCLC at diagnosis.

Much attention has been paid to the fibroblast growth factor receptor 2 (FGFR2), one of the FGFR family members that encodes transmembrane receptor tyrosine kinases (Silverman *et al*., 2021; Neumann *et al*., 2023). FGFR2 fusion genes have been found to have over 150 fusion partners (Silverman *et al*., 2021 and Goyal *et al*., 2023). A recent investigation found that truncating FGFR2's exon 18 (E18) is a strong driver mutation that may be a target for therapy (Zingg *et al*., 2022). There is evidence that the FGF family and FGFRs can be utilised to explain how HCC develops, progresses, and is treated. At least with regard to the angiogenic maintenance of tumours in patients with head cancer, FGF2 engaged in tumour angiogenesis to expedite tumour progression. It is thought that FGF2 has a major role in the development of HCC (Wang *et al*., 2021). In the current study, we thus used next-generation sequencing to evaluate a group of Egyptian patients with HCC for genetic variations in the FGFR2 and gene. Mutations were identified in 15 (71.4%) patients (Fig. 1). This high proportion might be explained by the study's short sample size and ethnic makeup.

Single nucleotide variations (SNVs) that occur in the coding areas of proteins alter the functional integrity of the protein and, as a result, raise the risk of developing a variety of diseases, including cancer. Because it necessitates extensive testing of the mutant gene, the screening of SNVs linked to certain phenotypes is concerning. One potential resolution could involve utilising computational tools to organise the mutations according to their functional attributes (Mahmood *et al*., 2022). When compared to the genomic control, there were twenty-six somatic mutations were detected, of these 22/26 (84.6%) were single nucleotide variants (SNVs), 1/26 (3.8%) was copy number variants (CNVs), 1/26 (3.8%) was multi nucleotide variant substitutions (MVNs) and 2/26 (7.8%) were insertion and deletion (INDELs). Among SNVs, 6/22 (27.3%) were non-synonymous, 4/22 (18.2%) were synonymous, and 12/22 (54.5%) were coding sequence variants. Concerning FGFR2 gene expression data analysis, there is predominance in transcript (52.4%) more than coding transcript (47.6%) (Fig. 1).

The demographic and clinicopathological features of HCC patients that are associated with the FGFR2 gene mutation are shown in Table (2). The mean ages of patients with the non-mutated type and mutant type were (66.0 ± 8.25 and 61.0 ± 9.24 respectively). Among studied patients, 13 (81.3%) of the mutated patients were males, compared to 3 (18.8%) females who were mutated. Additionally, the mean AFP value was 146.82 ± 246.69 in patients with the non-mutated type and 2978.22 ± 10579.01 in patients with the mutant type. The Clinical data of HCC Patients and the distribution of FGFR2

somatic mutations among them were summarized in Table (3).

In addition to the clinical data of HCC cases there is illustration of the distribution of the locus of FGFR2 mutations on the chromosome 10 among HCC cases and the classification of them to novel or existing mutations. A total of 6 non-synonymous variants were identified. In order to predict the impact of these variants on the protein function the Non-synonymous mutations in FGFR2 gene, we employed SIFT (http://sift.jcvi.org/www/SIFT_seq_submit2.html), and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2) softwares. SIFT scores identified as deleterious (intolerant) and tolerant (benign). Variants with PolyPhen values were classified to benign; 'Possibly Damaging', and 'Probably Damaging' (Table 4). The analysis of the pathogenicity of FGFR 2 gene, synonymous mutations is shown in Table (5). Missense SNPs, also known as non-synonymous SNPs (nsSNPs) or mutations, are very significant because they cause functional variation in human proteins by substituting amino acid residues. Previous studies have shown that nsSNPs account for around 50% of the mutations linked to a variety of genetic disorders (Feroz and Islam, 2023). With regard to the association between clinicopathological data and mutation, there was insignificant association between FGFR2 mutation and all studied variables.

According to Tripodi *et al*., (2020), amino acids (AAs) play a significant role in the proliferation and metabolism of primary liver cancer cells. Numerous AAs have been associated with the onset and progression of tumours, according to previous studies (Plewa *et al*., 2017; Hiraoka *et al*., 2019 and Liu *et al*., 2022). In this study, we analyzed AA changes in patients with HCC due to non-synonymous mutations in FGFR2 gene. In this study there were 6 non-synonymous. These non-synonymous mutations in FGFR2 gene were predicted by SIFT to be 3 deleterious and 3 tolerated variants, out of these variants 4 were benign, 1 were possibly damaging and 1 were Probably damaging when predicted by PolyPhen 2.

- The mutation (10: 123274722) was present in B Stage. This mutation causes amino acid change from (Asparagine to Proline), that is categorized as deleterious probably damaging mutation. This study's findings concur with those of Ding *et al*., (2020), who observed that increased proline biosynthesis boosted the proliferation of HCC cells.

- The (10: 123274732) mutation was present in A, B and C stages, this mutation categorized as tolerated benign mutation. This mutation causes amino acid change from (Isoleucine to Leucine). Leucine aminopeptidases (LAPs) were associated with advanced tumor stage and aggressive biological behavior, and thus a poor outcome (Ren *et al*., 2021).

- The mutation (10: 123279608_c.824A>C), which categorized as tolerated benign mutation. This mutation causes amino acid change from (Glutamate to Alanine). A study of HCC associated with HBV revealed a significant alanine upregulation. The rapid division of tumour cells and poor amino acid utilisation in HCC may be the cause of the overexpression of alanine amino acid (Khalil *et al*., 2021).

- The (10: 123279613_c.819C>A) mutation was present in C stage, this mutation categorized as deleterious benign. This mutation causes amino acid change from (Aspartate to Glutamate). Glutamate was significantly more abundant in HCC than in cirrhosis, according to the expression profiles of amino acids in advanced liver cirrhosis and HCC (Khalil *et al*., 2021).

- The mutation (10:123279615) mutation was present in B stage, this mutation categorized as tolerated possibly damaging cause amino acid change from (Aspartate to Asparagine). This study's findings concur with Bai *et al*., (2022), who showed that certain asparagine metabolism genes are essential for the development and prognosis of HCC.

- The mutation (10: 123279663 _c.939+30A>T) mutation was present in D stage, this mutation categorized as deleterious benign cause amino acid change from (Isoleucine to Valine). The serum and tissue valine level increase in HCC patients (Wu *et al*., 2022).

Conclusion

Number of mutations in FGFR2 have been identified, these mutations were non-Synonymous. Our work illustrates the association between genetic variants and clinicopathological characteristics and offers a fresh perspective on the genomic profiling of Egyptian HCC patients. At present, clinicians need to facilitate genetic testing as the use of NGS led to the discovery of several unique gene mutations in HCC. Larger patient cohorts are required to fully comprehend FGFR2 genetic alterations and their impact on the development of HCC. A large panel of genes could be ordered, considering the benefit for the patient and the cost for the public health care system. To fully explain genetic alterations in HCC, additional studies are needed, including whole exome sequencing.

SUMMARY

Background and objectives: The reliability of fibroblast tyrosine kinase receptor 2 (FGFR2) amplification as the biomarker for FGFR inhibitors in the hepatocellular carcinoma (HCC) is not satisfactory. There is urgent need to comprehensively characterize genetic aberrations of the FGFR2 gene. Simultaneously, we sought to determine the frequency of FGFR2 mutations as a potential tool to detect those alterations association with HCC development and

the distribution of the clinical-pathological features of HCC patients with FGFR2 mutations.

Patients and methods: Twenty-one patients with newly diagnosed and pathologically confirmed HCC were analyzed using a special Next generation sequencing panel (AmpliSeq). Genetic mutations of FGFR2 gene were identified and analyzed for correlations with clinical-pathological outcome.

Results: Recurrent mutations were observed in FGFR2 in 15 (71.4%) patients. When compared to the genomic control, there were twenty-six somatic mutations were detected, of these 22/26 (84.6%) were single nucleotide variants (SNVs), 1/26 (3.8%) was copy number variants (CNVs), 1/26 (3.8%) was multi nucleotide variant substitutions (MVNs) and 2/26 (7.8%) were insertion and deletion (INDELs). Among SNVs, 6/22 (27.3%) were non-synonymous, 4/22 (18.2%) were synonymous, and 12/22 (54.5%) were coding sequence variants. Concerning FGFR2 gene expression data analysis, there is predominance in transcript (52.4%) more than coding transcript (47.6%). The alterations ranged from deleterious to undefinable significance to tolerable deviations. No significant differences were observed between the mutation status of FGFR2 gene, and clinicopathological features of HCC patients.

Conclusion: By using bioinformatics, we concluded the roles of FGFR2 genetic variants in the diagnosis and prediction of the HCC development. Taken together, our data underscore to screen HCC patients for FGFR2 aberrations in oncology clinic.

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Table (1): Demographic and Clinicopathological characteristics of HCC patients.

Variables		HCC (n = 21)	
		No.	%
Smoking	Yes	2	9.5
	No	14	66.7
	Ex. smoker	5	23.8
Bilharzia	Yes	13	61.9
	No	8	38.1
Hepatic encephalopathy	Yes	0	0.0
	No	21	100.0
Family history	Yes	4	19.0
	No	17	81.0
Viral infection	HCV	18	85.7
	HBV	1	4.8
	NBNC	2	9.5
Comorbidities	DM	7	33.3
	HTN	3	14.3
Ascites	No	17	81.0
	Minimal	3	14.3
	Moderate	1	4.7
Portal Vein Invasion	Negative	18	85.7
	Positive	3	14.3
LN Metastasis	Negative	18	85.7
	Positive	3	14.3
Lung Metastasis	Negative	18	85.7
	Positive	3	14.3
Child PUGH class	A	16	76.2
	B	3	14.3
	C	2	9.5
Lesion Number	Single	10	47.6
	Multiple	11	52.4
Tumor size	Small (<3 cm)	5	23.8
	Large (>3 cm)	16	76.2
BCLC	A	7	33.3
	B	5	23.8
	C	9	42.9
	A + B	12	57.1
	C + D	9	42.9

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.

Table (2): Univariate FGFR2 mutation analysis of clinicopathological characteristics of HCC patients.

		FGFR 2				P-value
		No (n = 5)		Yes (n = 16)		
		No.	%	No.	%	
Age (years)	<60	1	20.0	7	43.8	^{FE}p=0.606
	≥60	4	80.0	9	56.2	
	Median (Min. – Max.)	63.0 (57.0 – 79.0)		60.50 (48.0 – 80.0)		0.294
	Mean ± SD.	66.0 ± 8.25		61.0 ± 9.24		
Gender	Male	5	100.0	13	81.3	^{FE}p=0.549
	Female	0	0.0	3	18.8	
BCLC	A	1	20.0	6	37.5	^{MC}p=0.665
	B	2	40.0	3	18.8	
	C	2	40.0	7	43.8	
	A + B	3	60.0	9	56.3	^{FE}p=1.000
	C + D	2	40.0	7	43.8	
Medical history	Bilharzias	3	60.0	10	62.5	^{FE}p=1.000
	Diabetes	4	80.0	3	18.8	^{FE}p=0.025*
	HTN	2	40.0	1	6.3	^{FE}p=0.128
Family history		2	40.0	2	12.5	^{FE}p=0.228
LN		1	20.0	2	12.5	^{FE}p=1.000
Metastasis		1	20.0	2	12.5	^{FE}p=1.000
Viral infection	HCV	4	80.0	14	87.5	^{FE}p=0.429
	HBV	0	0.0	1	6.25	^{FE}p=1.000
	NBNC	1	20.0	1	6.25	^{FE}p=0.429
Smoking	Non-smoker	2	40.0	12	75.0	^{MC}p=0.438
	Smoker	1	20.0	2	12.5	
	Ex-smoker	2	40.0	2	12.5	

Table (2): Cont''

P.S		0	0.0	2	12.5	^{FE}p=1.000
AFP	≤20	2	40.0	4	25.0	^{FE}p=0.598
	>20	3	60.0	12	75.0	
	Median (Min. – Max.)	46.10 (10.0 – 586.0)		41.10 (4.90 – 42443.0)		0.905
	Mean ± SD.	146.82 ± 246.69		2978.22 ± 10579.01		
Ascites	No	5	100.0	12	75.0	^{MC}p=0.648
	Mild	0	0.0	3	18.8	
	Moderate	0	0.0	1	6.2	
P.V		1	20.0	2	12.5	^{FE}p=1.000
Child-Pugh	A	5	100.0	11	68.8	^{MC}p=0.731
	B	0	0.0	3	18.8	
	C	0	0.0	2	12.5	
Lesions Number	Single	2	40.0	8	50.0	^{FE}p=1.000
	Multiple	3	60.0	8	50.0	
Lesions Size	Small (<3)	0	0.0	5	31.2	^{FE}p=0.278
	Large (≥3)	5	100.0	11	68.8	

SD: Standard deviation; MC: Monte Carlo; FE: Fisher Exact; p: p value for comparing between No and Yes; *: Statistically significant at p ≤0.05.

Table (3): The Clinical data of HCC Patients and the distribution of FGFR2 somatic mutations.

Pt. code	Age (yrs)	Gender	BCLC	Smoking No./ day	LN	Met	HCV	HBV	NBNC	P.S	Encephalopathy	Number	P.V	AFP	Chlid- pugh	Locus of Mutation	Mutation
HCC-1	60	M	C	5/day	No	No	No	No	Yes	Yes	No	*multiple*	No	5.5	B	*chr10:123279466*	*G>T/T*
HCC-2	68	F	B	No	No	No	Yes	No	No	No	No	*multiple*	No	50.4	A	*chr10:123279477*	*T>T/G*
																chr10:123279478	*T>T/C*
																chr10:123279615	*C>T/T*
HCC-3	52	M	B	No	No	No	Yes	No	No	No	No	*multiple*	No	42443	A	*chr10:123257952*	*10q26.13(123257952-123279713)x10*
																chr10:123274722	*C>C/G*
																chr10:123274727	*C>C/G*
																chr10:123274729	*G>G/A*
																chr10:123279619	*T>T/C*
HCC-4	50	F	B	No	No	No	Yes	No	No	No	No	*multiple*	No	16.8	A	*chr10:123257952*	*10q26.13(123257952-123279713)x5*
																chr10:123274732	*T>T/G*
																chr10:123279619	*T>T/C*
HCC-5	67	M	D	Ex 10 months	No.	Lung	Yes	No	No	No	No	*multiple*	No	22	C	*chr10:123257952*	*10q26.13(123257952-123279713)x9*
																chr10:123279477	*T>T/G*
																chr10:123279482	*A>A/G*
HCC-6	80	M	A	No	No	No	Yes	No	No	No	No	*single*	No	4.9	A	*chr10:123257952*	*10q26.13(123257952-123279713)x10*
																chr10:123257995	*T>T/G*
																chr10:123274732	*T>T/G*
																chr10:123279619	*T>T/C*
HCC-7	63	M	A	No	No	No	Yes	No	No	No	No	*single*	No	586	A	*chr10:123257995*	*T>T/G*
																chr10:123279619	*T>T/C*
HCC-8	65	M	C	No	No	No	Yes	No	No	Yes	No	*single*	No	69	B	*chr10:123279478*	*T>T/A*
																chr10:123279482	*A>A/G*
																chr10:123279613	*G>T/T*

Table (3): Cont"

																chr10:123279619	*T>T/C*
HCC-9	61	M	A	No	No	No	Yes	Yes	No	No	No	*multiple*	No	143	A	*chr10:123257952*	*10q26.13(123257952-123279713)x4*
																chr10:123257995	*T>T/G*
																chr10:123274732	*T>T/G*
																chr10:123279473	*T>T/C*
HCC-10	76	M	C	No	No	No	Yes	No	No	No	No	*multiple*	Yes	4370	B	*chr10:123274732*	*T>T/G*
																chr10:123279619	*T>T/C*
HCC-11	48	M	C	No	Yes	No	Yes	No	No	No	No	*multiple*	No	25.1	A	*chr10:123274829*	*A>A/C*
HCC-12	68	M	C	EX 18 years	Yes	Lung	Yes	No	No	No	No	*multiple*	No	72	A		*No mutation*
HCC-13	63	M	C	No	No	No	No	No	Yes	No	No	*single*	Yes	46.1	A		*No mutation*
HCC-14	79	M	B	No	No	No	Yes	No	No	No	No	*multiple*	No	10	A		*No mutation*
HCC-15	54	M	C	EX 15 Years	Yes	Lung	Yes	No	No	No	No	*multiple*	No	38	A	*chr10:123257952*	*10q26.13(123257952-123279713)x4*
HCC-16	67	M	A	No	No	No	Yes	No	No	No	No	*single*	No	22.7	A	*chr10:123257952*	*10q26.13(123257952-123279713)x3*
																chr10:123279456	*CC>TG/TG*
																chr10:123279458	*A>ATG/ATG*
HCC-17	59	M	A	Yes	No	No	Yes	No	No	No	No	*single*	No	65.23	A		*No mutation*
HCC-18	53	F	A	No	No	No	Yes	No	No	No	No	*single*	No	6.7	A	*chr10:123279688*	*T>T/C*
HCC-19	57	M	B	EX 1 Years	No	No	Yes	No	No	No	No	*multiple*	No	20	A		*No mutation*
HCC-20	53	M	D	No	No	No	Yes	No	No	No	No	*single*	Yes	62	C	*chr10:123257952*	*10q26.13(123257952-123279713)x10*
																chr10:123279463	*T>T/A*
																chr10:123279466	*G>G/T*
																chr10:123279467	*T>T/A*
																chr10:123279663	*T>T/C*
HCC-21	63	M	A	45Y, 10/d	No	no	Yes	No	No	No	No	*single*	No	325	A	*chr10:123274829*	*A>A/C*
																chr10:123279466	*G>G/A*

Table (4): Predictions, Clinical Significance of Non- synonymous FGFR2 mutations.

Position	Variation change	Exon	cDNA position	cDs position	Mutation predictors		Amino acid change	
					SIFT	Polyphen2	Old AA	New AA
chr10: 123274722	NA	9/18	1791	1199	deleterious	probably damaging	Arg	Pro
Chr10: 123274732	NA	9/18	1781	1189	tolerated	benign	Ile	Leu
Chr10: 123279608	c.824A>C	7/18	1416	824	tolerated	benign	Glu	Ala
Chr10: 123279613	c.819C>A	7/18	1411	819	deleterious	benign	Asp	Glu
Chr10: 123279615	NA	7/18	1409	817	tolerated	possibly damaging	Asp	Asn
Chr10: 123279663	c.939+30A>T	7/18	1361	769	deleterious	benign	Ile	Val

Table (5): Predictions, Clinical Significance of synonymous FGFR2 mutations.

Position	Coding variation	Exon	cDNA position	cDs position	Mutation predictors			Amino acid change
					Codons position	SIFT	Polyphen2	
Chr10: 123274727	NA	9/18	1786	1194	C1194G	NA	NA	L398L
Chr10: 123274829	NA	9/18	1684	1092	A1092C	NA	NA	P364P
Chr10: 123279619	NA	7/18	1405	813	T813C	NA	NA	G271G
Chr10: 123279625	c.807G>A	7/18	1399	807	C807T	NA	NA	V269V

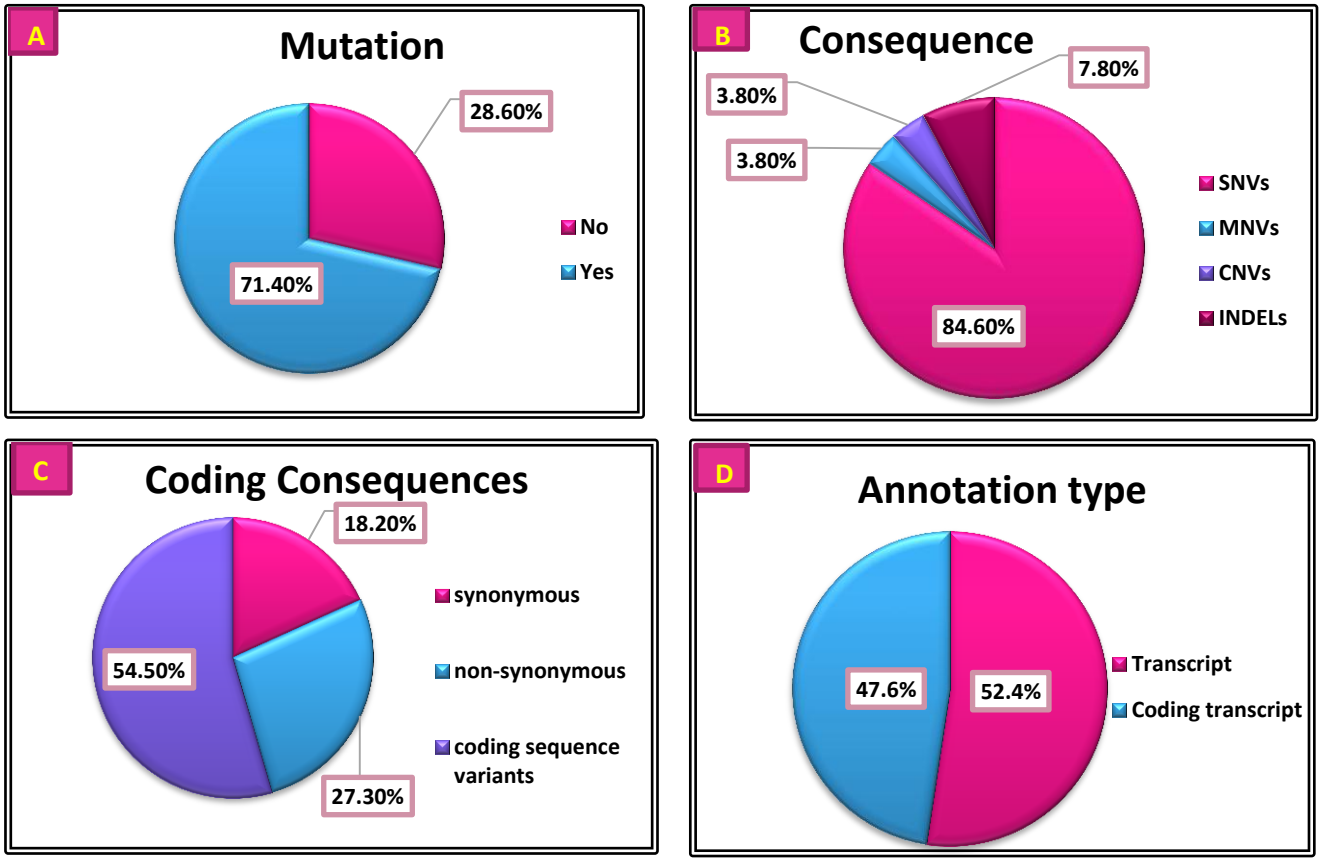

Fig. (1): Distribution of the studied cases according to (A): FGFR2 mutation Prevalence; (B): Consequence; (C): Coding consequence; (D): Annotation type.