DETECTION OF FMS-related tyrosine kinase-3 (FLT3) MUTATIONS PROFILE IN EGYPTIAN HEPATOCELLULAR CARCINOMA PATIENTS

MANAL O. EL HAMSHARY [1], MOHAMED AWAD[1*], ADEL E HEGAZY[2], RANDA M. TALAAT[1], MUSTAFA A. SAKR[1], MOHAMED K. KHALIFA[3], EHAB A. AHMED[4], GHADA M. NASR[1]

1 Department of Molecular Diagnostic and Therapeutic, Genetic Engineering and Biotechnology Research Institute, University of Sadat city, Egypt

2 Department of Plant Biotechnology, Genetic Engineering & Biotechnology Research Institute, University of Sadat city, Egypt

3Children Cancer Hospital, 57357, Egypt

4Chemistry Department, Faculty of Science, Cairo University, Egypt

*corresponding Auther:

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The World Health Organization (WHO) released its 2020 worldwide cancer statistics, and among all malignancies, liver cancer ranks third in death and sixth in incidence (Sung *et al*., 2021). Hepatocellular carcinoma (HCC) accounts for over 90% of all primary liver cancer cases. Other primary liver malignancies including intrahepatic cholangiocarcinoma follow (Chon *et al*., 2021 and Oh *et al*., 2023). Roughly 90% of cases of hepatocellular carcinoma have a known underlying cause; these include aflatoxin poisoning, chronic alcohol consumption, nonalcoholic fatty liver disease, hepatitis B and hepatitis C virus infections, and chronic viral hepatitis (Oh *et al*., 2023 and Pinheiro *et al*., 2024). Heterogeneity is a well-researched phenomena in HCC that results in variation in the cells, molecules, functions, and lineages. It is thought to be brought on by patients' varying genetic diversity and environmental factors (Safri *et al*., 2024).

Usually, a thorough diagnostic process that includes laboratory testing, imaging modalities, and physical examinations is used to identify HCC. Ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) are the main imaging tests used to diagnose liver cancer. To be sure of the diagnosis, a liver biopsy might be done. Alpha-fetoprotein

(AFP) testing and liver function tests are two other common laboratory tests used to diagnose liver cancer and track the disease's advancement (Feng and Zhao, 2024). Angeli-Pahim *et al*. (2023) state that the majority of patients with chronic liver disease are discovered at an advanced stage, making surgical intervention unfeasible.

A non-invasive marker of the presence of a tumour, circulating tumour DNA (ctDNA) is the fraction of cell-free DNA (cfDNA) released into the bloodstream after the necrosis or death of cancer cells. Commensurate with its use as a "liquid biopsy" for identifying cancerous genetic abnormalities, ctDNA has the capacity to be serially monitored for surveillance purposes. Next generation sequencing (NGS) technology has made it possible to identify the genetic events that cause hepatocarcinogenesis (Lyu *et al*., 2022). Only two to six driver mutations per tumour have a major impact on the tumor's evolution; the majority of mutations in each tumour are found in passenger genes (Caruso *et al*., 2021 and Yang *et al*., 2023). The initiation and progression of HCC is influenced by a number of signalling pathways, including WNT/β-catenin, RAS/RAF/MAPK, phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR), as well as the signalling linked to telomere maintenance, p53/cell cycle regulation, epigenetic modifiers, oxidative stress, and ubiquitin/proteasome degradation (Raja and Haq, 2022).

Out of all the human receptor tyrosine kinases, FLT3, or FMS-related tyrosine kinase-3, is one. FLT3 is primarily expressed on lymphoid progenitor cells, early myeloid cells, and haematopoietic stem cells. Under typical physiological circumstances, the growth factor FLT3-ligand (FL), which is secreted by haematopoietic and fibroblast cells in the bone marrow milieu, activates FLT3. Flavorod *et al*. (2023) reported that FL binding to FLT3 triggers signalling through PI3K, STAT5, and RAS, hence enhancing cell survival, differentiation, and proliferation. Rats' activation of FLT3 during liver regeneration raises the possibility that FLT3 aids in hepatocyte proliferation. According to Li *et al*. (2022) there is a close link between haematopoiesis and the foetal liver, and FLT3 plays a role in cellular proliferation and liver regeneration.

This study aims to determine the risk factors of HCC in Egyptian populations and determine the frequency, types and effect of FLT3 gene mutations by NGS-based cell-free DNA (cfDNA).

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 cir-

rhosis (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 x g 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 FLT3 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.

Statistical analysis

While data for categorical variables were given as frequencies and percentages, data for continuous variables were reported as mean ± Standard Deviation or

median (IQR). For continuous data, Mann-Whitney U tests were performed; for categorical data, the Chi-square test was employed to examine the relationship between the variables. The cutoff point for statistical significance was set at $P<0.05$. SPSS version 28 was used for the statistical analysis (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 FLT3 gene alterations were associated with the development of HCC.

In this study, males were predominant, representing 18 (85.7%) of the study population. The age of the studied HCC patients ranged from 48 to 80 years, 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. 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.

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). Males made up 85.7% of the HCC patients in the current study, with a male-to-female ratio of 6 to 1. 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.

The main causes of hepatocellular carcinoma (HCC) include HCV, which had been detected in 18 patients (85.75%); while one case had HBV (Fig. 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 this study, four HCC patients (19%) had a positive family history of cancers. Medical history of all study population had been reported and showed bilharziasis in 13 (61.90%) patients, diabetes in 7 (33.3%) patients and Hypertension in 3 (14.3%) patients. The smoking status showed that the majority of patients were non-smoker 14 (66.7%) (Fig. 1). According to Ramadan *et al*. (2021), 67.7% of Egyptian HCC patients had bilharzia antibodies. This result is consistent with their findings. 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).

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). 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% (Fig. 1). 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 (Fig. 1). 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. In this study, 52.4% of the HCC patients had multiple lesions. In 76.2% of the study population, most had large tumours (> 3) (Fig. 1). 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.

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 preva-

lence 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.

The FLT3 gene and protein expression was significantly decreased in specimens from patients with HCC compared with that in adjacent normal liver tissue. This reduced expression of FLT3 in HCC was attributed to the frequent FLT3 copy number losses. Notably, although the prognosis of patients with HCC and high FLT3 levels or copy number gains was poor, high FLT3 levels were significantly correlated with the improved overall survival (OS) of patients with HCC undergoing sorafenib treatment (Sun *et al*., 2020). Thus, we assessed a sample of HCC Egyptian patients for genetic variants in the FLT3 and gene using targeted sequencing in the current investigation. In the current study, 17 patients (81.0%) had FLT3 gene mutations. The study's small sample size and ethnicity may be related to this high proportion.

The single nucleotide variants (SNVs) ensuring in the coding regions of the proteins affect the functional integrity of the protein and, therefore, increase the susceptibility toward many diseases, including cancer. The screening of SNVs associated with specific phenotypes is a point of concern as it requires comprehensive testing of the mutated gene. A possible solution is prioritizing the mutations based on their functional characteristics using computational tools (Mahmood *et al*., 2022). When compared to the genomic control, there were 36 somatic mutations were detected, of these 29/36 (80.6%) were single nucleotide variants (SNVs), 2/36 (5.5%) were copy number variants (CNVs), 4/36 (11.1%) were multi nucleotide variant substitutions (MVNs) and 1/36 (2.8%) was insertions/deletion variants (INDELs). Among SNVs, 6/29 (20.7%) were synonymous, 8/29 (27.6%) were non-synonymous, and 15/29 (51.7%) were coding sequence variants (Fig. 2). Previous studies have shown that non-synonymous (nsSNPs) account for around 50% of the mutations linked to a variety of genetic disorders (Feroz and Islam, 2023).

The demographic and clinicopathological features of HCC patients that are associated with the FLT3 gene mutation are shown in Table (1). Additionally, the mean AFP value was 40.8 ± 20.5 in patients with the non-mutated type and 3011.1 ± 10234.5 in patients with the mu-

tant type. There is no significant association between FLT3 mutation and clinicopathological features. The Clinical data of HCC Patients and the distribution of FLT3 somatic mutations among them were summarized in Table (2).

In addition to the clinical data of HCC cases there is illustration of the distribution of the locus of FLT3 mutations on the chromosome 13 among HCC cases. A total of 8 non-synonymous variants were identified (Table 3). The analysis of FLT3 gene, 6 synonymous mutations is shown in Table 4. Previous studies have demonstrated that a variety of amino acids AAs are involved in the occurrence and development of tumors (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 FLT3 gene. In this study there were 8 non-synonymous mutations as follows:

-The mutation (13:28592615_ c.2530G>A) cause amino acid change from (Valine to Isoleucine). This study's findings concur with Jung *et al*., (2021), who observed that Isoleucine play prominent roles in tumor development and progression. On the other hand, recent reports described that BCAA (BCAA; leucine, isoleucine, and valine) suppressed the incidence of HCC (Takegoshi *et al*., 2017).

- The mutation (13:28608306_c.1750T>G) cause amino acid change from (Leucine to Glutamine). Glutamine metabolic reprogramming plays a pivotal role in HCC identification, proliferation, and progression (Ye *et al*., 2023).

- The mutation (13:28608306_c.1750T>G) cause amino acid change from (Serine to Alanine).

- The mutation (13:28608312_c.1744A>G) cause amino acid change from (Threonine to Alanine).

- The mutation (13:28608315_c.1741G>T) cause amino acid change from (Valine to Leucine). This result in contrary with Wu *et al*., (2022) who confirmed that serum Leucine level was lower in HCC than that in chronic hepatitis B.

- The mutation (13:28608336_c.1720A>C) cause amino acid change from (Serine to Arginine). The mutation (13:28610100_c.1390T>A) cause amino acid change from (Tryptophan to Arginine). This result in agreement with previous study which reported arginine as a novel second messenger-like molecule that reprograms metabolism to promote liver tumor growth (Mossmann *et al*., 2023).

- The mutation (13:28610095_c.1395G>C) cause amino acid change from (Lysine 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.

Conclusion

The results of this study shed light on the primary causative variables of HCC in Egypt, particularly HCV infection. Number of mutations in FLT3 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, including both confirmed and disproven mutations. The origin and progression of HCC are best understood because of these findings, which offer a new view.

SUMMARY

Background and objective: Hepatocellular carcinoma (HCC) is the third most common cancer globally and a major cause of mortality. Despite advancements in early treatment, advanced cases often have a poor prognosis due to high rates of recurrence. Understanding the disease's underlying mechanisms and associated genetic abnormalities is crucial for effective treatment. Recent research that sequenced all of the coding exons in HCC has provided fresh insight into the genetic characteristics of this cancer.

Patients and methods: In this cross-sectional study, 21 HCC Egyptian individuals were included and the FLT3 mutations in those individuals were detected using a special Next generation sequencing (NGS) panel (AmpliSeq). In addition, study the associations between these mutations and patient clinical characteristics.

Results: In all 21 patients who underwent FLT3 gene sequencing, mutations were identified in (81%) patients. When compared to the genomic control, there were 36 somatic mutations were detected, of these (80.6%) were single nucleotide variants (SNVs), Among SNVs, (20.7%) were synonymous, (27.6%) were non-synonymous, and (51.7%) were coding sequence variants.

Conclusion: It was concluded from this research that detection of numerous somatic mutations of FLT3 can assist in the etiology of HCC.

REFERENCES

Al-Haimi M., Hamid G. A. and Ahmed A. S., (2018). Hepatocellular carcinoma in Yemen: Epidemiology and clinical presentation. World J. Pharm. Med Res., 4:248-253.

Ali A. A. K., Gamal S. E., Anwar R., (2023). Assessment of clinico-epidemiological profile of Hepatocellular carcinoma in the last two decades. Egypt J. Intern. Med., 35: 18. https://doi.org /10.1186/s43162-023-00201-8.

Angeli-Pahim I., Chambers A., Duarte S. and Zarrinpar A., (2023). Current Trends in Surgical Management of Hepatocellular Carcinoma. *Can-*

cers. 15(22):5378. https://doi.org/10.3390/cancers15225378.

Bai J., Tang R., Zhou K., Chang, J., Wang H., Zhang Q., Shi J., and Sun C., (2022). An asparagine metabolism-based classification reveals the metabolic and immune heterogeneity of hepatocellular carcinoma. BMC medical genomics, 15(1): 222. https ://doi.org/10.1186/s12920-022-01380-z.

Borde T., Nezami N., Laage Gaupp F., Savic L. J., Taddei T., Jaffe A., Strazzabosco M., Lin M., Duran R., Georgiades C., Hong K. and Chapiro J., (2022). Optimization of the BCLC Staging System for Locoregional Therapy for Hepatocellular Carcinoma by Using Quantitative Tumor Burden Imaging Biomarkers at MRI. Radiology, 304(1), 228-237. https ://doi.org/10.1148/radiol.212426.

Caruso S., O'Brien D. R., Cleary S. P., Roberts L. R. and Zucman-Rossi J., (2021). Genetics of Hepatocellular Carcinoma: Approaches to Explore Molecular Diversity. Hepatology (Baltimore, Md.), 73 Suppl 1, 14-26. https://doi.org/10.1002/hep.31394

Chen C-F. and Chang H-C., (2023). Time trend and age-specific gender difference in the incidence of liver cancer from 2009 to 2018 in Taiwan. Adv. Dig. Med 10: 135-142. https://doi. org/10.1002/aid 2.13313.

Chon Y. E., Jeong S. W. and Jun D. W., (2021). Hepatocellular carcinoma statistics in South Korea. Clin. Mol. Hepatol., 27:512-514.

Deo R. K., Chitalkar P., Malla S., K..C. I., Karki B. and Thapa R., (2021). Epidemiology, Clinico-radiological Profile and Management of Hepatocellular Carcinoma in a Tertiary Care Center in Nepal. Med J. Shree Birendra Hosp, 20(1):6-11. Available-from:https://www.nepjol.info/index php /MJSBH / article/view/32256.

Elkenawy I., El-Bendary M., EL-Gilany A. and Shabana W., (2022). Characteristics of Hepatocellular Carcinoma in Egyptian Patients: A Single Center Pilot Study . Afro-Egyptian Journal of Infectious and Endemic Diseases, 12(4): 402-409. doi: 10.21608/aeji.2022.169214.1261.

Fathy Barakat E. M., AbdAlaziz K. M., Mahmoud El Tabbakh M. M. and Alden Ali M. K., (2021). Characteristics of Hepatitis B virus-induced Hepatocellular Carcinoma in Egyptian patients. QJM An Int. J. Med 114(Supplement_1). Available from: https://academic.oup.com/ qjmed/article/114/Supplement_1/hcab107.006/6379183.

Fedorov K., Maiti A., and Konopleva M., (2023). Targeting FLT3 Mutation in Acute Myeloid Leukemia: Current Strategies and Future Directions. Cancers, 15(8), 2312. https://doi.org/10.3390/ cancers15082312.

Feng F., and Zhao Y., (2024). Hepatocellular Carcinoma: Prevention, Diagnosis, and Treatment. Medical principles and practice: international journal of the Kuwait University, Health Science Centre, 1-10. Advance online publication. https://doi.org/ 10.1159/ 000539349.

Feroz T. and Islam M. K., (2023). A computational analysis reveals eight novel high-risk single nucleotide variants of human tumor suppressor LHPP gene. Egypt J. Med Hum. Genet., 24, 47. https://doi.org/10.1186/s43042-023-00426-w.

Ganeshan D., Szklaruk J., Kaseb A, Kattan A. and Elsayes K. M., (2018). Fibrolamellar hepatocellular carcinoma: multiphasic CT features of the primary tumor on pre-therapy CT and pattern of distant metastases. Abdom Radiol, 43(12):3340-8. Available from: http://link.springer.com/10.1007/s00261-018-1657-2.

Hiraoka N., Toue S., Okamoto C., Kikuchi S., Ino Y., Yamazaki-Itoh R., Esaki M., Nara S., Kishi Y., Imaizumi A., Ono N., and Shimada K., (2019). Tissue amino acid profiles are characteristic of tumor type, malignant phenotype, and tumor progression in pancreatic tumors. Scientific reports, 9(1), 9816. https://doi.org/10.1038/s41598-019-46404-4.

Jung M. K., Okekunle A. P., Lee J. E., Sung M. K., and Lim Y. J., (2021). Role of Branched-chain Amino Acid Metabolism in Tumor Development and Progression. Journal of cancer prevention, 26(4): 237-243. https://doi.org/10. 15430/JCP.2021.26.4.237.

Kaewdech A., Sripongpun P., Assawasuwannakit S., Wetwittayakhlang P., Jandee S., Chamroonkul N., and Piratvisuth T., (2023). FAIL-T (AFP, AST, tumor size, ALT, and Tumor number): a model to predict intermediate-stage HCC patients who are not good candidates for TACE. Frontiers in medicine 10, 1077842. https://doi.org/ 10.3389/fmed.2023.1077842.

Lai Y. L., Wang K. H., Hsieh H. P., and Yen W. C., (2022). Novel FLT3/AURK multikinase inhibitor is efficacious against sorafenib-refractory and sorafenib-resistant hepatocellular carcinoma. Journal of biomedical science, 29(1), 5. https://doi.org/10.1186 /s12929-022-00788-0.

Liao J. I., Ho S. Y., Liu P. H., Hsu C. Y., Huang Y. H., Su C. W., Hou M. C. and Huo T. I., (2023). Prognostic Prediction for Patients with Hepatocellular Carcinoma and Ascites: Role of Albumin-Bilirubin (ALBI) Grade and Easy (EZ)-ALBI Grade. Cancers (Basel), 15(3):753. doi: 10.3390/cancers15030753.

Liu D., Wen G., Song C., Ji L. and Xia, P., (2022). Amino acid profiles in the tissue and serum of patients with liver cancer. Open Medicine, 17(1): 1797-1802. https: //doi.org/10.1515/med-2022-0589.

Lopez-Lopez V., Gomez Ruiz A., Lopez-Conesa A., Brusadin R., Cayuela V., Caballero-Illanes A., Torres M. and Robles Campos R., (2020). Effects of primary hypertension treatment in the oncological outcomes of hepatocellular carcinoma. Ann Transl Med 8(14):844. Doi: 10.21037/atm.2020.04.40.

Lyu X., Tsui Y. M., Ho D. W. and Ng I. O., (2022). Liquid Biopsy Using Cell-Free or Circulating Tumor DNA in the Management of Hepatocellular Carcinoma. Cellular and molecular gastroenterology and hepatology, 13(6): 1611-1624. https://doi.org/10.1016/j.jcmgh.2022.02.008.

Mahmood M. S., Afzal M., Batool H., Saif A., Aqdas T., Ashraf N. M. and Saleem M. 92022). Screening of Pathogenic Missense Single Nucleotide Variants From LHPP Gene Associated With the Hepatocellular Carcinoma: An In silico Approach. Bioinformatics and biology insights 16, 11779322221115547. https://doi. org/10.1177/11779322221115547.

Mossmann D., Müller C., Park S., Ryback B., Colombi M., Ritter N., Weißenberger D., Dazert E., Coto-Llerena M., Nuciforo S., Blukacz L., Ercan C., Jimenez V., Piscuoglio S., Bosch F., Terracciano L. M., Sauer U., Heim M. H. and Hall M. N., (2023). Arginine reprograms metabolism in liver cancer via RBM39. Cell, 186(23): 5068-5083.e23. https:// doi.org/10.1016/j.cell.2023.09.011

Oh J. H., and Jun D. W., (2023). The latest global burden of liver cancer: A past and present threat. Clinical and molecular hepatology, 29(2): 355-357. https://doi.Org/ 10.3350/cmh.2023.0070.

Okeke E., Davwar P. M., Roberts L., Sartorius K., Spearman W., Malu A. and Duguru M., (2020). Epidemiology of Liver Cancer in Africa: Current and Future Trends. Seminars in liver disease, 40(2): 111-123. https://doi.org/10.1055/s-0039-3399566.

Pinheiro P. S., Jones P. D., Medina, H., Cranford H. M., Koru-Sengul T., Bungum T., Wong R., Kobetz E. N. and McGlynn K. A., (2024). In-

cidence of Etiology-specific Hepatocellular Carcinoma: Diverging Trends and Significant Heterogeneity by Race and Ethnicity. Clinical gastroenterology and hepatology: the official clinical practice journal of the American Gastroenterological Association, 22(3): 562-571.e8. https://doi.org/10.1016/j.cgh.2023.08.016.

Plewa S., Horała A., Dereziński P., Klupczynska A., Nowak-Markwitz E., Matysiak J. and Kokot Z. J., (2017). Usefulness of Amino Acid Profiling in Ovarian Cancer Screening with Special Emphasis on Their Role in Cancerogenesis. International journal of molecular sciences, 18(12): 2727. https://doi.org/10.3390/ijms18122727.

Que J., Lin C. H., Lin L. C. and Ho C. H., (2020). Challenges of BCLC stage C hepatocellular carcinoma: Results of a single-institutional experience on stereotactic body radiation therapy. Medicine 99(32), e21561. https://doi.org/ 10.1097/MD.0000000000021561.

Raja A., and Haq F., (2022). Molecular classification of hepatocellular carcinoma: prognostic importance and clinical applications. Journal of cancer research and clinical oncology, 148(1): 15-29. https://doi.org/10.1007/s00432-021-03826-w.

Ramadan A., EL Ebidy G., Elzaafarany M., Galal A., Ibrahem M. and Ali D., (2021). Characterization of hepatocellular carcinoma in Mansoura University Hospitals: A case-control study of risk factors. Medical Journal of Viral Hepatitis, 6.1(1): 38-45. doi: 10.21608/mjvh.2021.211713.

Safri F., Nguyen R. and Zerehpooshnesfchi S., (2024). Heterogeneity of hepatocellular carcinoma: from mechanisms to clinical implications. Cancer Gene Ther. https://doi.org/ 10.1038/s41417-024-00764-w.

Sarma M., Padma S., Pavithran P., Somasundaram V. H. and Sundaram P. S., (2021). Extrahepatic metastases of hepatocellular carcinoma on 18F FDG PET CT. Journal of the Egyptian National Cancer Institute 33(1), 36. .https://doi.org/10.1186/s43046-021-00086-0

Sayiner M., Golabi P. and Younossi Z. M., (2019). Disease Burden of Hepatocellular Carcinoma: A Global Perspective. Dig. Dis. Sci., 64(4):910-917.

Shen C., Jiang X., Li M., Luo Y. and (2023). Hepatitis Virus and Hepatocellular Carcinoma: Recent Advances. Cancers 15(2):533. https://doi.org/10.3390/cancers15020533.

Sun W., Li S. C., Xu L., Zhong W., Wang Z. G., Pan C. Z., Li J., Jin G. Z., Ta N., Dong W., Liu D., Liu H., Wang

H. Y., Ding J. and (2020). High FLT3 levels may predict sorafenib benefit in hepatocellular carcinoma. Clin. Cancer Res. 26(16):4302-12.

Sung H., Ferlay J., Siegel R. L., Laversanne M., Soerjomataram I., Jemal A. and Bray F., (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: a Cancer journal for Clinicians, 71(3): 209-249. https://doi.org/10.3322/caac.21660

Takegoshi K., Honda M., Okada H., Takabatake R., Matsuzawa-Nagata N., Campbell J. S., Nishikawa M., Shimakami T., Shirasaki T., Sakai Y., Yamashita T., Takamura T., Tanaka T., and Kaneko S., (2017). Branched-chain amino acids prevent hepatic fibrosis and development of hepatocellular carcinoma in a non-alcoholic steatohepatitis mouse model. Oncotarget, 8(11): 18191-18205. https://doi.org/10.18632 /oncotarget.15304.

Thokerunga E., Kisembo P. and FangFang H., (2023). Serum Midkine for AFP-negative hepatocellular carcinoma diagnosis: a systematic review and meta-analysis. Egypt Liver Journal. 13, 25. https://doi.org/10.1186/ s43066-023-00259-7.

Wu T., Zheng X., Yang M., Zhao A., Xiang H., Chen T., Jia W. and Ji G., (2022). Serum Amino Acid Profiles Predict the Development of Hepatocellular Carcinoma in Patients with Chronic HBV Infection. ACS omega, 7(18): 15795-15808. .https://doi.org/ 10.1021/acsomega.2c00885.

Yang T. H., Chan C., Yang P. J., Huang Y. H. and Lee M. H., 2023. Genetic Susceptibility to Hepatocellular Carcinoma in Patients with Chronic Hepatitis Virus Infection. Viruses, 15(2): 559. doi:10.3390/v15020559.

Yang Z., Liu J., Xue F., Zhang L., Xue H., Wu Y., Bai S., Du F., Wang X., Deng W., Song C. and Wang, K., (2023). Genomic landscape of Chinese patients with hepatocellular carcinoma using next-generation sequencing and its association with the prognosis. Annals of hepatology, 28(2): 100898. https://doi.org/10.1016/ j.aohep.2023.100898.

Ye Y., Yu B., Wang H. and Yi F., (2023). Glutamine metabolic reprogramming in hepatocellular carcinoma. Frontiers in molecular biosciences, 10: 1242059. https: //doi.org/10.3389/fmolb.2023.1242 059.

Zhang H., Spencer K, Burley S. K. and Zheng X. F. S., (2021). Toward improving androgen receptor-

targeted therapies in male-dominant hepatocellular carcinoma. Drug Discov Today. 26 (6):1539-46. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1359644621000672.

Zhao S., Wang M., Yang Z., Tan K., Zheng D., Du, X. and Liu L., (2020). Comparison between Child-Pugh score and Albumin-Bilirubin grade in the prognosis of patients with HCC after liver resection using time-dependent ROC. Annals of translational medicine 8(8): 539. https://doi.org/10.21037/atm.2020.02.85.

Table (1): Univariate FLT3 mutation analysis of clinicopathological characteristics of HCC patients.

Variables		FLT3				p
		No (n = 4)		Yes (n = 17)		
		No.	%	No.	%	
Age (years)	<60	0	0.0	8	47.0	1.000
	≥60	4	100.0	9	53.0	
	Median (Min. – Max.)	60 (48-68)		63 (50-80)		0.434
	Mean ± SD.	59.0 ± 7.5		62.9 ± 9.0		
Gender	Male	4	100.0	14	82.4	0.629
	Female	0	0.0	3	17.6	
BCLC	A	0	0.0	7	41.2	0.897
	B	1	25.0	4	23.5	
	C	3	75.0	4	23.5	
	A + B	1	25.0	11	64.7	1.000
	C + D	3	75.0	6	35.3	
Medical history	Bilharzias	2	50.0	11	64.7	0.391
	Diabetes	4	100.0	3	17.6	0.088
	HTN	1	25.0	2	11.8	0.489
Family history		1	25.0	3	17.6	0.281
LN		2	50.0	1	5.9	0.415
Metastasis		1	25.0	2	11.8	0.489

Table (1): Cont.						
Viral infections	HCV	3	75.0	15	88.2	1.000
	HBV	0	0.0	1	5.9	1.000
	NBNC	1	25.0	1	5.9	1.000
Smoking	Non-smoker	1	25.0	13	76.5	0.397
	Smoker	1	25.0	2	11.8	
	Ex-smoker	2	50.0	2	11.8	
P.S		0	0.0	2	11.8	1.000
AFP	≤20	0	0.0	5	29.4	1.000
	>20	4	100.0	12	70.6	
	Median (Min. – Max.)	35.6 (20-72)		44.2 (4.9-42443)		0.576
	Mean ± SD.	40.8 ± 20.5		3011.1 ± 10234.5		
Ascites	No	4	100.0	13	76.5	0.260
	Mild	0	0.0	3	17.6	
	Moderate	0	0.0	1	5.9	
P.V		1	25.0	2	11.8	0.489
Child-Pugh	A	4	100.0	12	70.6	0.415
	B	0	0.0	3	17.6	
	C	0	0.0	2	11.8	
Lesions Number	Single	1	25.0	8	47.0	1.000
	Multiple	3	75.0	9	53.0	
Lesions Size	Small (<3)	0	0.0	5	29.4	0.532
	Large (≥3)	4	100.0	12	70.6	

SD: Standard deviation**;** p: p value for comparing between **No** and **Yes; *:** Statistically significant at p ≤0.05.

Table (2): The Clinical data of HCC Patients and the distribution of FLT3 somatic muttions.

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	chr13:28592579	*13q12.2(285925*
HCC-2	68	F	B	No	No	No	Yes	No	No	No	No	*multiple*	No	50.4	A	*chr13:28592579*	*13q12.2(285925*
																chr13:28602322	*T>T/G*
HCC-3	52	M	B	No	No	No	Yes	No	No	No	No	*multiple*	No	42443	A	*chr13:28592579*	*13q12.2(285925*
																chr13:28602322	*T>T/G*
																chr13:28608313	*C>G/G*
																chr13:28608328	*T>T/G*
																chr13:28610100	*A>A/T*
																chr13:28610173	*A>A/G*
HCC-4	50	F	B	No	No	No	Yes	No	No	No	No	*multiple*	No	16.8	A	*chr13:28592579*	*13q12.2(285925*
HCC-5	67	M	D	Ex 10 months	.	Lung	Yes	No	No	No	No	*multiple*	No	22	C	*chr13:28602322*	*T>T/G*
																chr13:28608328	*T>T/G*
																chr13:28610173	*A>A/G*
HCC-6	80	M	A	No	No	No	Yes	No	No	No	No	*single*	No	4.9	A	*chr13:28602322*	*T>T/G*
																chr13:28608291	*A>A/C*
																chr13:28608328	*T>T/G*
																chr13:28610113	*T>T/G*
HCC-7	63	M	A	No	No	No	Yes	No	No	No	No	*single*	No	586	A	*chr13:28592579*	*13q12.2(285925*
HCC-8	65	M	C	No	No	No	Yes	No	No	Yes	No	*single*	No	69	B	*chr13:28608316*	*C>T/T*
																chr13:28608328	*T>T/G*
HCC-9	61	M	A	No	No	No	Yes	Yes	No	No	No	*multiple*	No	143	A	*chr13:28592579*	*13q12.2(285925*
																chr13:28602322	*T>T/G*
																chr13:28608328	*T>T/G*

Table (2): Cont'

HCC-10	76	M	C	No	No	No	Yes	No	No	No	No	*multiple*	Yes	4370	B	*chr13:28592579* *chr13:28608328* *chr13:28610113*	*13q12.2(285925* *T>T/C* *T>T/A*
HCC-11	48	M	C	No	Yes	No	Yes	No	No	No	No	*multiple*	No	25.1	A		*No mutation*
HCC-12	68	M	C	EX 18	Yes	Lun	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	*chr13:28592612* *chr13:28592615* *chr13:28592618* *chr13:28592628* *chr13:28592651*	*T>G/G* *C>T/T* *CA>CG/TG* *A>G/G* *A>G/G*
HCC-15	54	M	C	EX 15 Years	Yes	Lun g	Yes	No	No	No	No	*multiple*	No	38	A	*chr13:28608277* *chr13:28610134*	*A>A/G* *A>A/G*
HCC-16	67	M	A	No	No	No	Yes	No	No	No	No	*single*	No	22.7	A	*chr13:28608328* *chr13:28608336*	*T>T/G* *T>T/C*
HCC-17	59	M	A	Yes	No	No	Yes	No	No	No	No	*single*	No	65.23	A	*chr13:28608303* *chr13:28608330* *chr13:28608333* *chr13:28608336*	*AG>AG/CA* *G>G/GA* *GG>GG/AC* *T>T/G*
HCC-18	53	F	A	No	No	No	Yes	No	No	No	No	*single*	No	6.7	A	*chr13:28592579* *chr13:28592621* *chr13:28608301*	*13q12.2(285925* *A>A/G* *T>T/C*
HCC-19	57	M	B	EX 1	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	*chr13:28592584* *chr13:28602326* *chr13:28602330* *chr13:28602335* *chr13:28610095*	*G>G/C* *CAC>AGG/AGG* *CC>GT/GT* *A>T/T* *C>G/G*
HCC-21	63	M	A	45Y, 10/d	No	no	Yes	No	No	No	No	*single*	No	325	A	*chr13:28592579* *chr13:28608306* *chr13:28608312* *chr13:28608328* *chr13:28610095*	*13q12.2(285925* *A>A/C* *T>T/C* *T>T/G* *C>C/G*

Table (3): Identification of FLT3 Non- synonymous mutations.

Position	variant freq	Coding	Amino acid change	
			Old A.A	New
chr13:28592615	0.04	c.2530G>A	Val	Ile
chr13:28602335	0.04	c.2033T>A	Leu	Gln
chr13:28608306	0.04	c.1750T>G	Ser	Ala
chr13:28608312	0.04	c.1744A>G	Thr	Ala
chr13:28608315	0.04	c.1741G>T	Val	Leu
chr13:28608336	0.08	c.1720A>C	Ser	Arg
chr13:28610095	0.08	c.1395G>C	Lys	Asn
chr13:28610100	0.04	c.1390T>A	Trp	Arg

Table (4): Identification of Synonymous Somatic Mutations in FLT3 gene.

Position	Variation Change	Coding
chr13:28592612	0.04	c.2533A>C
chr13:28592628	0.04	c.2517T>C
chr13:28592651	0.04	c.2494T>C
chr13:28608277	0.04	c.1779T>C
chr13:28608328	0.32	c.1728A>C
chr13:28610134	0.04	c.1356T>C

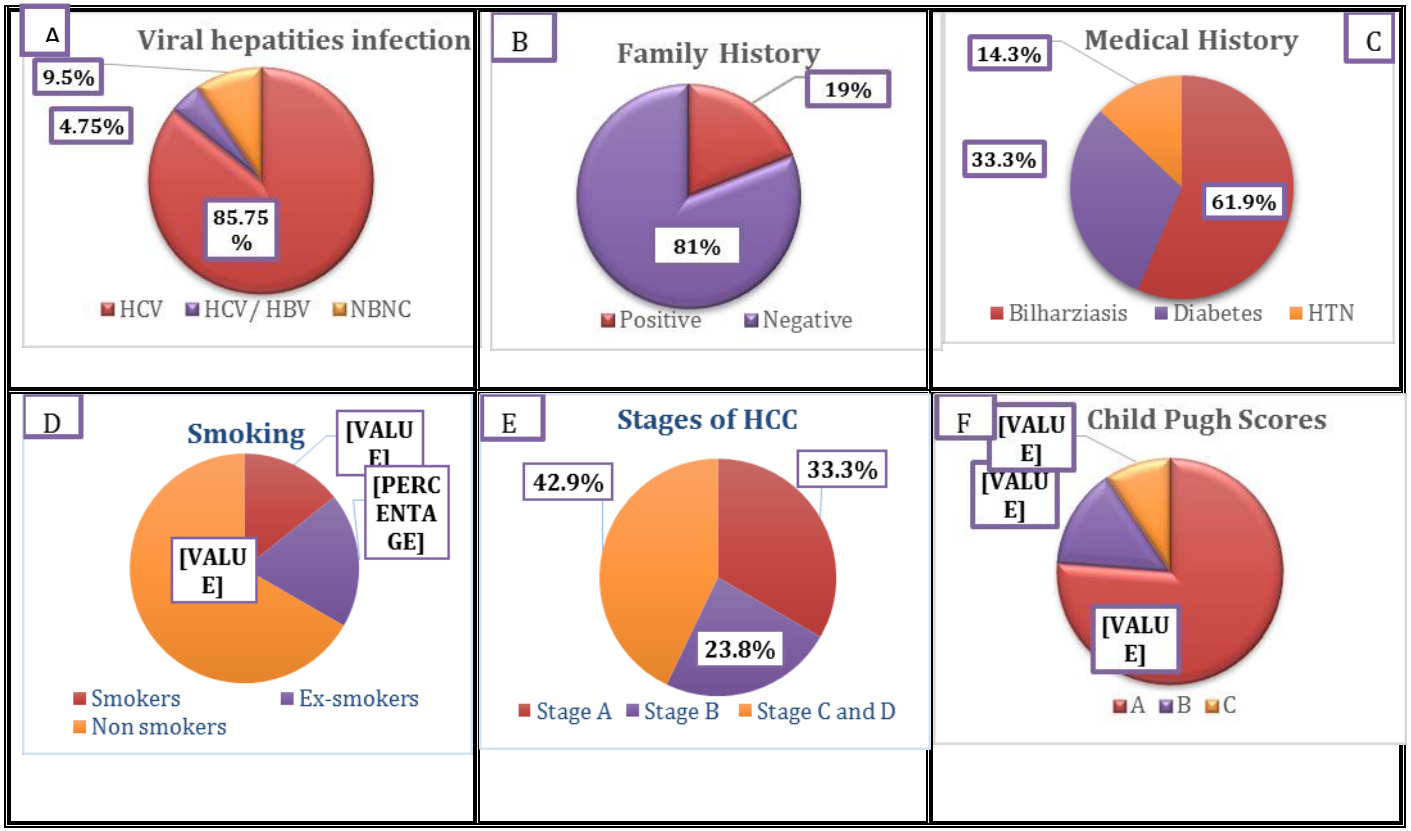
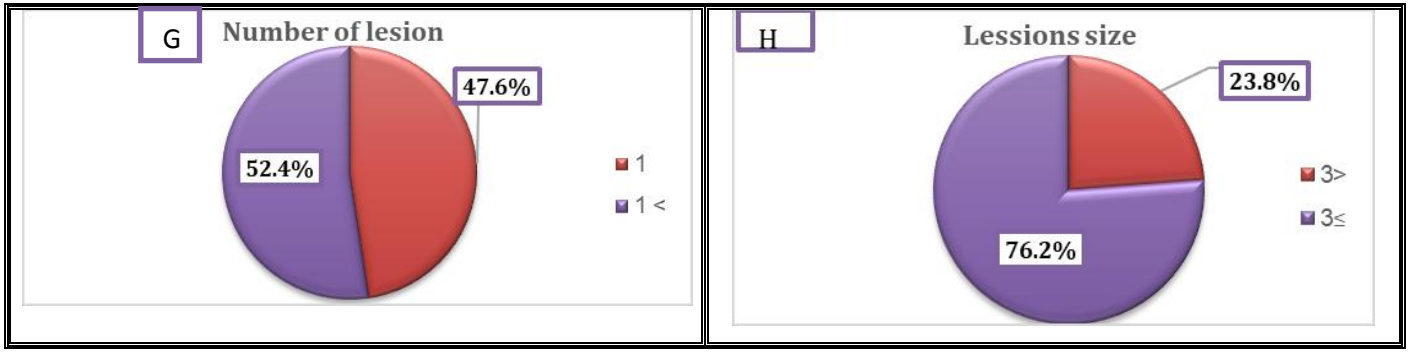


Fig. (1): Distribution of risk factors and tumour characteristics in HCC patients. A. Viral hepatitis infection; B. Family History; C. Medical history for bilharziasis, diabetes, and Hypertension; D. Smoking status; E. BCLC classification; F. Child-Pugh; G. Tumor number; H. Tumor size.

Fig. (1): Cont'



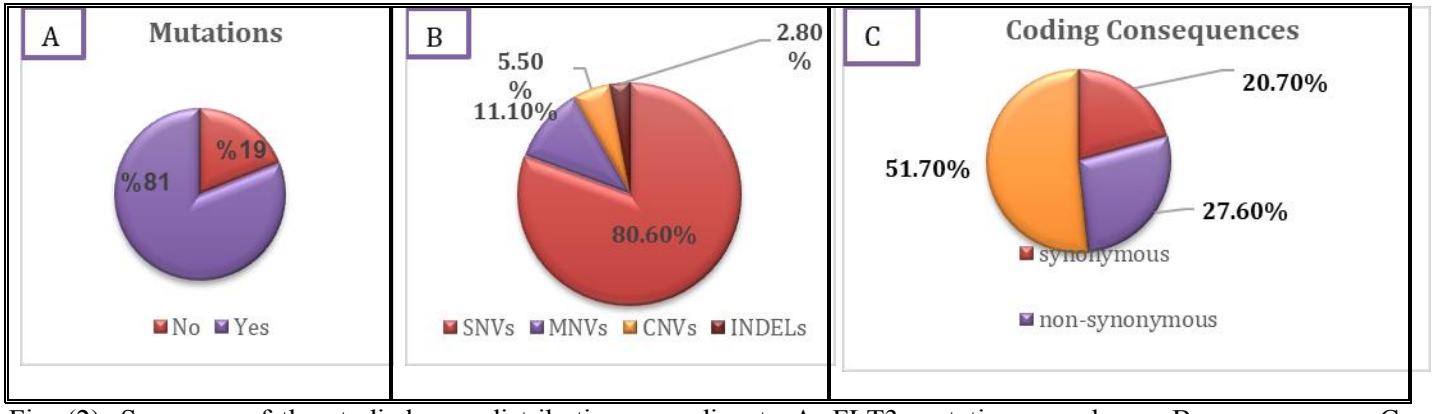


Fig. (2): Summary of the studied case distribution according to A. FLT3 mutation prevalence; B. consequence; C. coding consequence.