BIOPSY AS A NONINVASIVE METHOD FOR THE DETECTION OF *JAK3* SOMATIC MUTATIONS IN HCC EGYPTIAN PATIENTS BY NGS

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Liver diseases are a significant cause of mortality and morbidity throughout the world, causing two million deaths per year worldwide (3.5% of all death), with 50% related to cirrhosis complications and 50% associated with Hepatocellular Carcinoma (HCC) and viral hepatitis infections **(**Asrani *et al*., 2019**)**. Liver disease incidence and prevalence have increased significantly over the years with varying evolutions of etiologies (Wong *et al*., 2018).

HCC is the most common neoplasm in the liver, accounting to >80% of all primary liver cancers worldwide (Yang *et al*., 2019). It is reported to be the 4th common cause of cancer death with a poor prognosis with a 5-year survival rate of 6.9% (Yapali *et al.*, 2018). There is excellent global variation in the prevalence of HCC worldwide, with the highest prevalence reported in Eastern Asia and Sub-Saharan Africa (almost 85% of cases) (Sharafi *et al*., 2019).

Liquid biopsy has become a promising alternative non-invasive procedure, allowing for the isolation and detection of cancer-derived subcellular components released in biological fluids such as blood. Thus, it

is possible to overcome the difficulties in obtaining tissue biopsies, as in HCC. In addition, several circulating biomarkers can be detected in liquid biopsies, such as cell-free DNA, tumor cells, microRNAs, and exosomes which are secretory vesicles containing nucleic acids and proteins. Hence, liquid biopsy has become an appealing source of biomarkers for several applications in cancer, such as diagnosis, prognosis, and prediction of treatment response (Shigeyasu *et al*., 2017).

Numerous investigations have shown that cancer-associated molecular features and tumor-specific genetic changes are present in cfDNAs from cancer patients and tumor cells can release DNA into peripheral blood (Thierry *et al*., 2014).

Four paralogous genes JAK1, JAK2, JAK3, and TYK2 encode JAKs. These tyrosine kinases are drawn to cytokine receptors, where they phosphorylate essential substrates, most significantly STAT proteins that bind DNA and control gene expression, to transmit signals. T-cell prolymphocytic leukaemia (T-PLL), natural killer/T-cell lymphoma, adult T-cell lymphoma (ATLL), and cutaneous T-cell lymphoma (CTCL) have all been linked to JAK3 mutations (NKTL) (McGirt *et al*., 2015).

Janus kinase 3 (JAK3) is involved in cytokine receptor-mediated intracellular signal transduction. *JAK3* mutation has been identified in ESCC and HCC (Hu *et al*., 2016 and Lu *et al*., 2015) while germline activating mutation of *JAK3* has been detected in 6.7% (62/932) of patients with non-small cell lung cancer (NSCLC) (Li *et al*., 2017).

Both *JAK1* and *JAK3* mutations had been described in hematologic malignancies and have proven to be oncogenic in various assays (Forbes *et al*., 2010).

Our hypothesis was the possible role of cfDNA sequencing in carcinogenesis, so the aim of our study was to evaluate the frequent deleterious somatic mutations of *JAK3* among HCC Egyptian patients by using NGS technology to understand and interpret genetic alterations.

SUBJECTS AND METHODS

1. Patients

The present study was conducted at the Molecular Diagnostics Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), Elsadat City University, between October 2019 and January 2021.

This study comprised 21 hepatocellular cancer patients from the oncology clinic of the National Liver Institute-Menoufia University-Egypt. To exclude all germ line mutations of these patients, their cfDNA targets were compared to the genome of 3

healthy people with no malignancies. The study got approved by the Ethics Committee of the National Liver Institute (NLI IRB procedure 00232/2020, Dec.2020). All of the 21 cases of our study suffered only from HCC without any other types of cancers.

2. Methods

The medical records of all patients included medical and relevant history, clinical data, clinical examination, tumor staging, chest X-ray, triphasic computed tomography (CT), and complete laboratory tests (Complete liver function tests (Cobas 6000 analyzer[c501 module, Roche diagnostics]), HCV antibody, HBV-sAg, and AFP serum level (Cobas 6000 analyzer [e601 module, Roche diagnostics]) and Real-time PCR for HCV RNA (Abbott m2000rt).

Sampling

Six ml blood was collected on 3 EDTA tubes that were gently mixed, and then blood was centrifuged by cooling centrifuge. Plasma containing cfDNA fragments was separated from blood and frozen at -80ºC for cell-free DNA extraction by (QIAamp DSP Virus spin Kit, Cat.No.61704).Two ml blood was collected on an EDTA tube for genomic extraction by (PureLink™ Genomic DNA Mini Kit, Cat.No.K1820-00**)**.

Next-generation sequencing

The next step was DNA libraries preparation (Ion AmpliSeq™ Library Kits 2.0, Cat.No.4480441) and Ion AmpliSeq HiFi Master Mix (Ion AmpliSeq™ Library kit 2.0, Thermo Fisher Scientific, Inc.) were used for amplification process. Then qPCR with the ion library TaqMan® Quantitation Kit (Thermo Fisher Scientific, Inc.) was used to quantify the amplified libraries. In the next step: Ion PGM™ Hi‑Q™ View OT2 Kit (Thermo Fisher Scientific, Cat.No.A29900) was used to prepare Enriched, template-positive Ion PGM™ Hi-Q™ View Ion Sphere™ Particles (ISPs). Then the Ionsphere quality control kit (Thermo Fisher Scientific, Inc.) was utilized to ensure that between 10 and 30% of template-positive ISPs were produced.

JAK3 somatic mutations were detected by generation sequencing (iontorrent platform), using (Ion PGM™ Hi-Q™ View Sequencing Kit, Cat.No.A30044). Ion Personal Genome Machine System (Ion Torrent) (PGM™; Life Technologies) were used to load the enriched template ISPs onto Ion 316™ chips and be sequenced as instructed by the protocol (Morishita *et al.*, 2017) where Real‑time measurements of hydrogen ions produced during JAK3 DNA fragments replication by The Ion PGM™ Sequencer were done.

Bioinformatics

ThermoFisher website was used to upload BAM files produced from the sequencer to the Ion reporter server version 5.10. The paired normal, and the ion amplseq custom panel system analyzed tumor samples. Torrent Suite software (version 3.6.2; Thermo Fisher Scientific, Inc.) was utilized to join the unprocessed data to Human Genome version 19 (hg19). Coverage Analysis plug-in (version 3.6; Thermo Fisher Scientific, Inc.) was used for coverage analysis. The cut-offs were: quality>20, coverage of the average base was >500× reads, the allele frequency >10% and total uniformity > 80%. Variant Caller plug-in (version 3.6; Thermo Fisher Scientific, Inc.) was used to identify mutations. Integrative Genome Viewer (IGV) from the Broad Institute was utilized to verify each detected mutation (www. broadinstitute.org) (Thorvaldsdottir *et al*., 2012).

Statistics

Using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp): Chi-square test Fisher's Exact or Monte Carlo correction, Mann Whitney test and Student t-test to explore the relation between *JAK3* mutations and clinicopathological features. P-value < 0.05 was used to determine the statistically significant difference.

RESULTS AND DISCUSSION

Study population

As shown in Tables (1 &2): the study was conducted in 18 males and three females. Thirteen patients were above or equal to 60 years old and eight patients were under the age of 60. By Barcelona score, seven patients (33.3%) were stage A, five patients (23.8%) were stage B and nine patients (42.9%) staged C and D (Fig. 1). Among the studied cases, 15 patients had AFP > 20ng/ml, 19 were HCV infected, one was HBV infected and two were not infected by HCV nor HBV. Tables (1&2) also illustrate the correlation between *JAK3* genetic mutation and other clinicopathological features.

Profiling of *JAK3* mutations

(Germline vs. somatic and synonymous vs. nonsynonymous mutations)

The present study showed that *JAK3* was mutated in 10 patients from all 21 (47.6%) (Table 1). By comparing all gene mutations in cfDNA with the control (genomic control) (paired sample analysis), there were 26 mutations: 13/26 (50%) were germline and 13/26 (50%) were somatic. Among somatic mutations only one mutation was synonymous 1/13 (7.7%), six mutations were nonsynonymous 6/13 (46.2%), 5 mutations 5/13 (38.5%) were in the intron region, and only one mutation (7.7%)

was unavailable.

By using variant effect predictor (VEP) analysis of SNV mutations, the highest type was of missense variants (75%) followed by synonymous variants (25%) (Fig. 2). Only there was one synonymous mutation found in the exonic region of the mutant *JAK3* while according to the reading of prediction tools like SIFT (Kumar *et al*., 2009), and polyphen (Adzhubei *et al*., 2013) which predicts that some non-synonymous mutations may cause critical changes in protein (Table 3), a total of 6 nonsynonymous variants were recognized, one of them was reported as presented variants either in single nucleotide polymorphism database (dsSNP) or (COSMIC).

Clinicopathological features

Table (1) illustrates the correlation between the mutated *JAK3* and the clinicopathological features, where it shows there was no statistical significance between them upon the p-value where it was more than 0.05 for all.

***JAK3* genetic mutations effects**

The Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway was supposed to be involved in the frequently altered mechanism in HCC. *JAK3* mutations detected in our study pointed to the probability of involvement of (JAK-STAT) pathway in HCC development.

Some nonsynonymous mutations of *JAK3* were deleterious mutations in advanced stages (Table 3) such as patient 3 (stage A) who has one deleterious (novel) mutation, and another not predicted novel mutation, patient 15 (stage C) has one deleterious mutation (novel) and another not predicted novel mutation, finally patient 21 (stage D) has a (novel) mutation that was deleterious. All those deleterious mutations may have an effect on the etiology and development of HCC among patients.

According to Yang and Roberts (2010), HCC makes up around 85% of liver cancer cases and is distinguished by a pathophysiology that is very heterogeneous, an aggressive clinical course, and a poor prognosis. Compared to those for other malignancies, the risk factors for HCC are more well-defined. Chronic hepatitis B virus (HBV), hepatitis C virus (HCV), chronic excessive alcohol use, aflatoxin B1 (AFB1) exposure, and nonalcoholic fatty liver disease are among the risk factors (NAFLD) (Venook *et al*., 2010).

The malignant transformation of normal hepatocytes happens through a multistep biological process known as hepatocarcinogenesis, which is widely acknowledged to be extremely complex and influenced by a number of factors,

including genetic and epigenetic changes. (Yoon *et al*., 2018).

HCC has cancer-specific DNA alterations that are associated with malignancies caused by mutation, methylation, and gene integrity which can be used to diagnose the disease (Jung *et al*., 2010). cfDNA mutations are valuable diagnostic markers with a sensitivity of 65% and specificity of 100%. cfDNA expression levels are unrelated to age, gender, or AFP levels, signifying an advantage in detecting early tumors (Xiong *et al*., 2019).

Potential clinical utilities of cfDNA/ ctDNA have been and are being investigated for detecting HCC, disease monitoring, and prognostication (Tran *et al*., 2021).

In our study on a sample of Egyptian HCC patients, the targeted sequencing was used to detect *JAK3* genetic variations, and some earlier existing and novel genomic variations with known or unknown biological importance were found. Observed nonsynonymous mutations in *JAK3* variations and their predicted effects on protein activity might play a role in HCC development.

Previous studies (Pesu *et al*., 2008 and Elliott *et al*., 2011), JAK3 is preferentially expressed in hematopoietic cells, as opposed to JAK1, JAK2, or Tyk2 members of the non-receptor tyrosine kinase family, and JAK3 mutations have been found in leukemic individuals and cell lines. Two non-synonymous JAK3 mutations were found in patients with HCC in a previous investigation. (LU *et al*., 2015).

JAK3 mutations detected in the current study might play a minimal role in HCV-associated hepatocarcinogenesis. A study conducted by (Hin Tang *et al*., 2020) showed that many intracellular signaling pathways contribute to hepatocarcinogenesis, including the JAK/STAT pathway, which has normal roles in regulating cell proliferation, survival, and differentiation. However, the deregulation of JAK/STAT signaling is observed in many cancers and contributes to various oncogenic effects.

In the present study, the identified nonsynonymous mutations in *JAK3* had a deleterious effect on protein functions and are reported in the COSMIC database, while the rest of the nonsynonymous mutations in *JAK3* were not described in COSMIC, which require additional studies due to their possible impact in HCC initiation.

Statistically, there was no statistical significance between clinical features and mutated genes because all P values in the present study were above 0.05 (Table 1). The cases within this work were classified into three

subgroups based on Barcelona clinic liver cancer classification (BCLC); with stage A representing early HCC, stage B representing intermediate cases, and stage C&D representing advanced HCC. Among cases of stage A from (1 to 7): case 2 (male) and case 6 (female) had no fault in their *JAK3*. The two cases are infected by HCV but not HBV (Table 4).

Case 3, male 61 years old with HCV and HBV infection and AFP was 143 ng/ml and two liver lesions had one synonymous mutation, which was deleterious with probably damaging effects. Case 10 in stage B is a woman, 50 years old, with multiple liver lesions. She had two existing mutations and one novel mutation (chr19:17945671_T/G) with the deleterious effect, which might have a role in hepatocarcinogenesis.

Advanced HCC cases: 13, 14 16, 17, 18, and 19 with stage C (BCLC) (Table 4) had no mutations in their *JAK3*. This explains that these cases might have other mutated genes or were subjected to other causes for the development of HCC.

Case 15 with stage C is an old man with 76 years old, not a smoker, has no metastasis, no LN but had HCV infection. His Child-Pugh was B, and AFP level was 4370 ng/ml. He had multiple liver lesions Table (4). This case had 2 new mutations in JAK3 gene. (chr19:17947991_T/G) is nonsynonymous with deleterious probably damaging effect Table (3). The other novel one was the intron mutation. Case 17 was a diabetic man, 68 years old, stopped smoking from 18 years, lung metastasis, no LN, HCV infected, with 3 lesions in liver. He had no mutations in *JAK3* (Table 4).

Case 21, stage D, was a man, 53 years old, diabetic, infected with HCV but not HBV, with moderate ascites, C child-pugh, one liver lesion with size a of 8.5 * 7.8 cm (Table 4). He had one nonsynonymous mutation (chr19:17947994_C/T) which is a deleterious new mutation and benign (Table 3).

SUMMARY

One of the most prevalent malignancies in the world, hepatocellular carcinoma (HCC), has a high fatality rate. Noninvasive biomarkers are desperately needed to help in HCC screening and early diagnosis. Next-generation sequencing has advanced, and genetic indicators are now the mainstay of cancer detection. Early HCC diagnosis now focuses on genetic indicators such circulating tumour DNA in peripheral blood.

JAK3 is a member of the non receptor tyrosine kinase family, the members of which are able to bind to various cell surface receptors and are

important in cytokine induced signal transduction.

JAK3 mutations were not significantly associated with an increased risk of HCC in the Egyptian population. However, it could have a probable role in the pathogenesis of liver cell failure, HCC development, and prognosis, as the present study identified several novel genes involved in HCC using NGS.

A small sample size (21 cases) is considered one of the weak spots of our study. SO, we recommend that this study will be conducted with a larger cohort in the future to completely understand *JAK3* genetic alterations and their association with HCC development.

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Table (1): Correlation of clinicopathological features and mutations of JAK3 in HCC patients:

clinicopathological features	N	JAK3				P-value
		Wild type (n = 11)		Mutant (n = 10)		
		No.	%	No.	%	
Gender						
Male	18	10	90.9	8	80.0	0.586
Female	3	1	9.1	2	20.0	
Age (years)						
<60	8	5	45.5	3	30.0	0.659
≥60	13	6	54.5	7	70.0	
BCLC1						
A	7	4	36.4	3	30.0	1.000
B	5	2	18.2	3	30.0	
C & D	9	5	45.5	4	40.0	
AFP						
≤20	6	4	36.4	2	20.0	0.635
>20	15	7	63.6	8	80.0	
Bilharziasis						
No	8	5	45.5	3	30.0	0.659
Yes	13	6	54.5	7	70.0	
Diabetes						
No	14	6	54.5	8	80.0	0.361
Yes	7	5	45.5	2	20.0	
HTN						
No	18	9	81.8	9	90.0	1.000
Yes	3	2	18.2	1	10.0	
Family history						
No	17	9	81.8	8	80.0	1.000
Yes	4	2	18.2	2	20.0	
Smoking						
No	14	6	54.5	8	80.0	0.558
Smoker	3	2	18.2	1	10.0	
Ex-smoker	4	3	27.3	1	10.0	

Table (2): Correlation of clinicopathological features and mutations of JAK3 in HCC patients.

clinicopathological features	No	JAK3				P-value
		Wild type (n = 11)		Mutant (n = 10)		
		No.	%	No.	%	
LN						
No	18	8	72.7	10	100.0	0.214
Yes	3	3	27.3	0	0.0	
Metastasis						
No	18	9	81.8	9	90.0	1.000
Yes	3	2	18.2	1	10.0	
HCV						
No	2	2	18.2	0	0.0	0.476
Yes	19	9	81.8	10	100.0	
HBV						
No	20	11	100.0	9	90.0	0.476
Yes	1	0	0.0	1	10.0	
NBNC						
No	19	9	81.8	10	100.0	0.476
Yes	2	2	18.2	0	0.0	
P.S						
No	19	10	90.9	9	90.0	1.000
Yes	2	1	9.1	1	10.0	
Encephalopathy						
No	21	11	100.0	10	100.0	–
Yes	0	0	0.0	0	0.0	
P.V						
No	18	10	90.9	8	80.0	0.586
Yes	3	1	9.1	2	20.0	
Ascites						
No	17	10	90.9	7	70.0	0.378
Mild	3	1	9.1	2	20.0	
Moderate	1	0	0.0	1	10.0	
Child-pugh						
A	16	10	90.9	6	60.0	0.291
B	3	1	9.1	2	20.0	
C	2	0	0.0	2	20.0	
Number of lesions						
1	10	5	45.5	5	50.0	1.000
>1	11	6	54.5	5	50.0	
Size of lesions						
<3	5	3	27.3	2	20.0	1.000
≥3	16	8	72.7	8	80.0	

Table (3): Effects of nonsynonymous somatic mutations of *JAK3* on HCC patients using SIFT and PolyPhen.

	Pt. ID	Age	Gender	BCLC	AFP	C - P	locous of muation	mutation	E/N	Mut. type	SIFT	PolyPhen
Group I (Stage A)	HCC-1	80	M	A	4.9	A	chr19:17948066	A>A/C	N	intron	-------	-------
	HCC-2	63	M	A	586	A		no mutation				
	HCC-3	61	M	A	143	A	chr19:17947991	T>T/G	N	missense	deleterious	probably damaging
							chr19:17948066	A>A/C	N	intron	-------	-------
	HCC-4	67	M	A	22.7	A	chr19:17945671	T>T/C	N	missense	deleterious	possibly damaging
	HCC-5	59	M	A	65.23	A		no mutation				
	HCC-6	53	F	A	6.7	A		no mutation				
	HCC-7	63	M	A	325	A	chr19:17948066	A>A/C	N	intron	-------	-------
Group II (Stage B)	HCC-8	68	F	B	50.4	A	chr19:17945696	C>C/T	E	missense	tolerated	benign
	HCC-9	52	M	B	42443	A	chr19:17945671	T>T/C	N	missense	deleterious	possibly damaging
	HCC-10	50	F	B	16.8	A	chr19:17945671	T>T/G	N	missense	deleterious	possibly damaging
							chr19:17948074	A>A/C	N	intron	-------	-------
	HCC-11	79	M	B	10	A		no mutation				
	HCC-12	57	M	B	20	A		no mutation				
Group III (Stages C&D)	HCC-13	60	M	C	5.5	B		no mutation				
	HCC-14	65	M	C	69	B	chr19:17947991	no mutation				
	HCC-15	76	M	C	4370	B	chr19:17947991	T>T/G	N	missense	deleterious	probably damaging
							chr19:17948066	A>A/C	N	intron	-------	-------
	HCC-16	48	M	C	25.1	A		no mutation				
	HCC-17	68	M	C	72	A		no mutation				
	HCC-18	63	M	C	46.1	A		no mutation				
	HCC-19	54	M	C	38	A		no mutation				
	HCC-20	67	M	D	22	C	chr19:17945671	T>T/C	N	missense	deleterious	possibly damaging
							chr19:17948066	A>A/C	N	intron	-------	-------
	HCC-21	53	M	D	62	C	Chr19:17947994	C>C/T	N	missense	deleterious	benign

Pt.ID: Patient identification. **C-P:** Child-pugh score. **E:** Existing. **N:** Novel. **Mut. type:** Mutation type. **SIFT:** scale-invariant feature transform. **PolyPhen:** Polymorphism Phenotyping.

Table (4) Distribution of *JAK3* mutations among HCC patients and their clinical data.

Pt. ID	Age	Gender	BCLC	Smoking No./ day	LN	Metas	HCV	HBV	NBNC	PS	AFP	Ascites	PV	C-P	Lesions		locus of muations	mutation	E/N
															Number	Size of largest lesion (cm)			
HCC-1	80	M	A	No	No	No	Yes	No	No	0	4.9	No	No	A	1	3.9*3.5cm	Chr19:17948066	A>A/C	N
HCC-2	63	M	A	No	No	No	Yes	No	No	0	586	No	No	A	1	9*8.2 cm		no mutation	
HCC-3	61	M	A	No	No	No	Yes	Yes	No	0	143	No	No	A	2	3*3CM 7/2/2018	chr19:17947991	T>T/G	N
																	Chr19:17948066	A>A/C	N
HCC-4	67	M	A	No	No	No	Yes	No	No	0	22.7	No	No	A	1	11.3*7cm	chr19:17945671	T>T/C	N
HCC-5	59	M	A	Yes	No	No	Yes	No	No	0	65.23	No	No	A	1	7*7cm		no mutation	
HCC-6	53	F	A	No	No	No	Yes	No	No	0	6.7	No	No	A	1	1.2cm		no mutation	
HCC-7	63	M	A	45Y, 10/d	No	no	Yes	No	No	0	325	No	No	A	1	6*3.5*3 cm	Chr19:17948066	A>A/C	N
HCC-8	68	F	B	No	No	No	Yes	No	No	0	50.4	No	No	A	2	4* 5 cm	Chr19:17945696	C>C/T	E
HCC-9	52	M	B	No	No	No	Yes	No	No	0	42443	No	No	A	Multiple	5*4cm	chr19:17945671	T>T/C	N
HCC-10	50	F	B	No	No	No	Yes	No	No	0	16.8	No	No	A	Multiple	1.5 cm	chr19:17945671	T>T/G	N
																	chr19:17948074	A>A/C	N
HCC-11	79	M	B	No	No	No	Yes	No	No	0	10	No	No	A	3	4*3cm		no mutation	
HCC-12	57	M	B	EX 1 Years	No	No	Yes	No	No	0	20	No	No	A	Multible	4.7*4.2cm - 4.0		no mutation	
HCC-	60	M	C	5/day	No	No	No	No	Yes	1	5.5	Mild	No	B	2	2.7*2 cm		no muta-	

Table (4): Cont.'

13																	tion	
HCC-14	65	M	C	No	No	No	Yes	No	No	1	69	Mild	No	B	1	3.8cm 3/4/2016		no mutation	
HCC-15	76	M	C	No	No	No	Yes	No	No	0	4370	Mild	Yes	B	Multiple	3*3 cm	chr19:17947991	T>T/G	N
																	Chr19:17948066	A>A/C	N
HCC-16	48	M	C	No	Yes	No	Yes	No	No	0	25.1	No	No	A	2	5.9*5.4*5		no mutation	
HCC-17	68	M	C	EX 18 years	Yes	Lung	Yes	No	No	0	72	No	No	A	3	3.7*3.2		no mutation	
HCC-18	63	M	C		No	No	No	No	Yes	0	46.1	No	Yes	A	1	11.5*8cm		no mutation	
HCC-19	54	M	C	EX 15 Years	Yes	Lung	Yes	No	No	0	38	No	No	A	Multiple	1.9*1.5cmMRI		no mutation	
HCC-20	67	M	D	Ex 10 months	No	Lung	Yes	No	No	0	22	No	No	C	3	1.8 cm	chr19:17945671	T>T/C	N
																	Chr19:17948066	A>A/C	N
HCC-21	53	M	D	No	No	No	Yes	No	No	0	62	Mod.	Yes	C	1	8.5*7.8 cm	Chr19:17947994	C>C/T	N

Pt.ID: Patient identification. **LN:** Lymph node. **Metas:** Metastasis. **PS:** Performance status. **PV:** Portal vein. **C-P:** Child-pugh score. **E:**Existing. **N:**Novel.

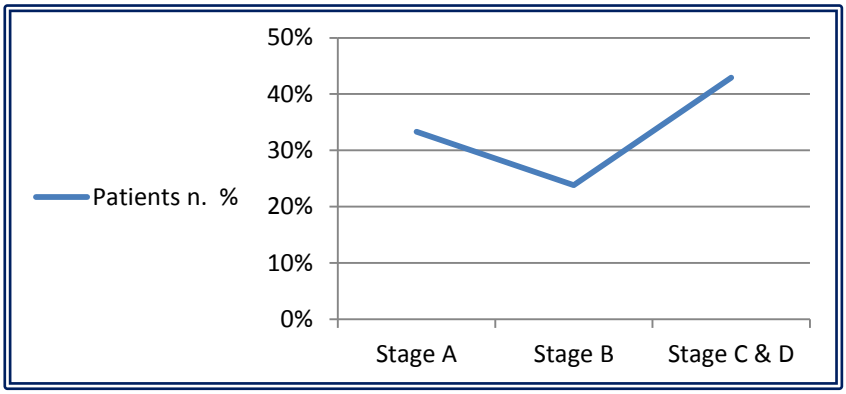

Fig. (1):Classification of HCC patients regarding BCLC staging System

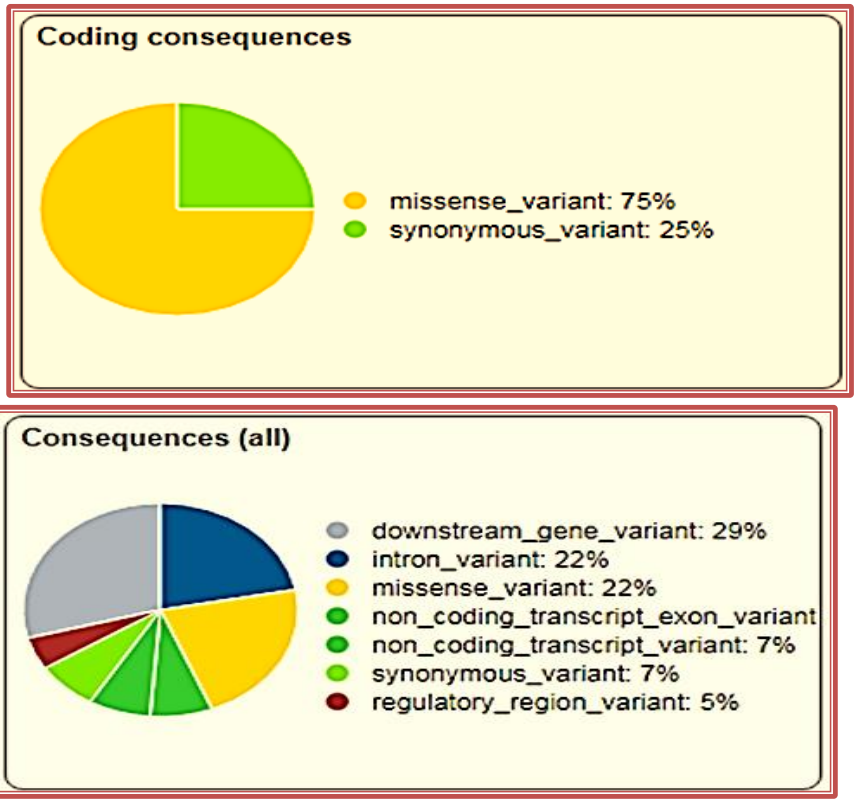

Fig. (2): *JAK3* somatic mutations among studied HCC patients.