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Single Nucleotide Polymorphism of ERCC1 Gene in Patients with Non-Small Cell Lung Cancer and its Relation to the Response to Platinum Chemotherapy

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ABSTRACT

Background: Non-small cell lung cancer (NSCLC) accounts for eighty five percent of lung cancer cases. Among drugs most commonly used are platinum-based chemotherapy as cisplatin and carboplatin & 3rd generation chemotherapy. Excision Repair Cross Complementing Group 1 (ERCC1) Gene is one of members of nucleotide excision repair pathway. It causes inhibition in the action produced by platinum and third generation chemotherapy. So, the produced DNA repair will be resistance to these drugs. Single nucleotide polymorphism in ERCC1 impairs this function and this may help in prediction of response to platinum-based chemotherapy.

Aim of the Work: This work aimed to study association among single nucleotide polymorphism of ERCC1 rs11615 in studied cases with non-small cell lung cancer and the response to platinum-based chemotherapy as to reduce exposure of chemotherapy side effect.

Materials & Methods: research was done on 50 NSCLC patients. Thirty of them were non-responders to platinum-based chemotherapy & other 20 were responders based on RECIST criteria. Detection of the ERCC1 (T/C) polymorphism by real-time PCR was done for all patients' groups.

Results: In responders' group, 19 patients (95%) had wild type homozygous CC genotype & 1patient (5%) had heterozygous TC genotype. In non-responders' group, 29 patients (96.7%) had wild type homozygous CC genotype and one patient (3.3%) had TC genotypes. There was no significant statistical variation observed among responders' & non-responders' groups regarding genotype frequencies (x^2= 0.8, p>0.05).

Conclusion: Our findings did not support existence of significant link among ERCC1 rs 11615 polymorphism & response to platinum-based chemotherapy in advanced cases of NSCLC.

Keywords: ERCC1- Lung cancer – Platinum-based chemotherapy.

INTRODUCTION

Behind prostate cancer in men & breast cancer in females, lung cancer is the 2nd most common cancer diagnosed in both genders. It is leading reason for cancer-related mortality worldwide, & its prevalence is rising rapidly. It accounts for twenty seven percent of all cancer deaths worldwide [(1)]. Lung cancer is classified into 2 histological types: small cell lung cancer & non-small cell lung cancer [(2)]. Non-small cell lung cancer accounts for eighty five percent of all cases of lung cancer. Squamous cell carcinoma, adenocarcinoma, & large cell carcinoma are 3 subtypes of NSCLC [(3)]**.** Common of the studied cases are diagnosed at progressive stages mainly (3 & 4) at time of presentation. Treatment options for NSCLC depend mainly on the stage at diagnosis and include: surgical resection, chemotherapy often along with radiotherapy. In early stages (0, I, II), surgical resection is possible. Therefore, chemotherapy & radiotherapy play dominant role in treatment of NSCLC [(4).]

Eastern Cooperative Oncology Group Performance Status (ECOGPS) score is used to evaluate performance status of lung cancer studied cases. It is classified into six grades (grade zero to five). Grade zero is fully operational, while grade five is dormant. ECOGPS score is used to quantify cancer studied cases' functional status & is important factor in determining prognosis in malignant conditions. When planning trials to research new treatment technique, researchers all over world consider ECOGPS [(5)]. Patients with ECOGPS 1 score are fully ambulatory & can perform light work, whereas studied cases with ECOGPS 2 score are ambulatory but cannot perform any work activities. Studied cases with ECOGPS 2 make up sizable proportion of cancer population [(6)].

Chemotherapy plan for lung cancer often consists of drug combination. Among drugs most commonly used, platinum based chemotherapy as cisplatin and carboplatin in addition to 3rd generation drugs as vinorelbine and gemcitabine. These agents act by disrupting the genetic material integrity by causing crosslinking of DNA, which results in inhibiting DNA repair and DNA synthesis in cancer cells and leads to cancer cells apoptosis [(3)].

Studied cases are classified into: Responder patients that further were subdivided into patients with complete response with disappearance of all target lesions and patients with partial response with more than thirty percent reduction of all target lesions. Non-responder patients that further subdivided into patients with progressive disease with more than or equal twenty percent rise from smallest sum of diameters recorded & five mm absolute rise over lowest sum and patients with stable disease with neither sufficient shrinkage to qualify for complete response nor sufficient rise to qualify for progressive disease [(3)].

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Efficacy of platinum-based chemotherapy is limited by chemoresistance. Classifying the patients' response to the chemotherapy can protect those patients from such harmful effects of chemotherapy and improve clinical outcomes [7].

ERCC 1 Gene is located on long arm of chromosome 19q at position 13.32. In physiological conditions, this gene produces protein that functions in nucleotide excision repair pathway & essential for repairing DNA lesions like those induced by UV light & formed by electrophilic compounds including cisplatin [8]. ERCC1 gene is member of nucleotide excision repair pathway that inhibits action produced by platinum and third generation chemotherapy. Thus, the produced DNA repair will cause resistance to platinum-based chemotherapy. Single nucleotide polymorphism in ERCC1 impairs this activity & can help to expect response to platinum-based chemotherapy & classify those studied cases into responders and non-responders [7]. Goal of this research was to look into relationship among ERCC1 single nucleotide polymorphism (19007 T>C) (N118N, rs11615) & reaction to platinum-based chemotherapy in non-small cell lung cancer studied cases in order to avoid unnecessary exposure to chemotherapy's toxic effects.

PATIENTS & METHODS

Research was done in Oncology Department, Ain Shams University Hospitals. It was conducted on 50 patients. It started from December 2019 till December 2021. Studied cases were eligible if they had histologically confirmed NSCLC, were over eighteen years old, had adequate hematologic function, adequate renal function (creatinine level 1.5 mg/dL), & adequate liver function. All participants received one of the following platinum-based regimens: cisplatin/gemcitabine regimens, cisplatin/taxol regimens (seventy five $mg/m^2$of cisplatin on day one & 175 $mg/m^2$of taxol. All chemotherapeutic drugs were given intravenously, & studied cases were treated for 3 to 6 cycles, with four-cycle average. There had been no thoracic radiotherapy. Every 3 cycles, computed tomography scan was used to evaluate tumor response. Response Assessment Criteria in Solid Tumors were used to assess responses. All enrolled studied cases were followed up on until end of December 2021. PFS was described as period between start of treatment & disease progression & death from any cause.

Exclusion criteria: Subjects with NSCLC who are not Egyptians, studied cases with progressive NSCLC who received any chemotherapy other than platinum, patients with severe complications due to chemotherapy hematology toxicity, febrile neutropenia, infection & thrombocytopenia related to bleeding, acute and chronic infections, patients with liver dysfunction, renal dysfunction, organ failure & brain metastasis.

Subjects were classified into two groups: Responders' group (Group I, n=20): This group had 20 studied cases who were diagnosed as NSCLC. It included 15 men & five women with a mean years old of 54.6 ranged from 40 to 76 years. According to RECIST, patients who showed complete response & partial remission (more than thirty percent reduction of all target lesions) were considered as good responders.

Non-Responders' Group (Group II, n=30): This group included 30 patients who were diagnosed as NSCLC. It included 29 males & one woman with years old of 61.9 ranged from 48 to 76 years. According to RECIST, patients who presented stable disease & progressive disease (more than or equal twenty percent rise from smallest sum of diameters recorded & five mm absolute rise over lowest sum) were regarded as non-responders.

All studied cases in this research had full history taken, including their gender, years old, tobacco use, & family history of cancers, followed by clinical examination, histopathological diagnosis of NSCLC & TNM classification, radiological studies like computed tomography of chest & abdomen, & laboratory investigations like complete blood picture (CBC), liver & kidney function tests Furthermore, for all individuals, single nucleotide polymorphism of ERCC1 gene rs11615 was noticed using real-time polymerase chain reaction method. TaqMan real time PCR kit from Thermo Scientific was used to identify ERCC1 polymorphism (rs11615). Method was carried out in two steps: extraction of genomic DNA from peripheral blood leucocytes in EDTA whole blood sample, followed by amplification of extracted DNA & allelic discrimination using real time PCR.

a) <u>Genomic DNA extraction:</u>

Gene JET whole blood genomic DNA purification mini kit was used to extract high-quality genomic DNA from whole blood in quick & efficient manner. Extraction method involves digesting samples with proteinase K in supplied lysis solution. Following that, lysate was mixed with ethanol & loaded onto purification column, where DNA binds to silica membrane. Impurities were effectively eliminated from column by washing it with ready wash buffer. Genomic DNA is then eluted with elution buffer under low ionic strength conditions.

b) <u>DNA amplification and real-time PCR allelic discrimination assays:</u>

The extracted DNA was amplified using TaqMan universal master mix & ready-made TaqMan SNP genotyping assay kit for rs11615 supplied by Thermo

scientific. TaqMan genotyping Master Mix, forward & reverse primers, & 2 TaqMan minor groove binder Probes are added to reaction mixture containing genomic DNA. Proximity of quencher dye to reporter dye suppressed reporter fluorescence when probe was intact. 2 TaqMan minor groove binder probes consist of target specific oligonucleotides with reporter dye at 5' end of each probe: a-VIC dye l linked to 5' end of Allele 1 probe. B-fluorescein amidites dye (5- & 6-carboxyfluorescein) l linked to Allele 2 probe's 5' end. At 3'end of each probe, non-fluorescent quencher is added.

Only probes that were hybridized to target were cleaved by AmpliTaq Gold DNA Polymerase, UP. Cleavage splits reporter dye from quencher dye, raising reporter's fluorescence. As result, fluorescence signal produced by PCR amplification suggests, which alleles are present in sample. Minor groove binder raises melting temperature without raising probe length, allowing shorter probes to be designed. Shorter probes resulted in greater Tm variation among matched & mismatched probes, allowing for precise allelic discrimination.

The context sequence for rs11615 was

Forward:

5’ CGGTTCCGAGGTGAGTGCAGTCATC -3’

Reverse:

5’ GAGGGAGAGAAGATGCTGGCTTGGC -3’

The presence to the absence of the SNP and the allelic discrimination was established according to the type of the emitted fluorescence of either of the reporter dyes or both at the same time as shown in table (1).

Table (1): Relation among fluorescence signals & sequences in sample

Fluorescence Increase	Indication
VIC dye fluorescence only (green)	Homozygosity for allele 1
6FAM dye fluorescence only (blue)	Homozygosity for allele 2
Fluorescence signals for both dyes	Heterozygosity for allele 1-allele 2

Ethical approval:

This study followed tents of Helsinki Declaration. All research protocols were approved by Ain Shams University's Institutional Ethical Committee. As result, prior to any intervention, all participants provided written informed consent. Writers have strictly adhered to ethical standards (including plagiarism, data fabrication, & double publication).

Statistical Analysis

Data were coded, entered, & analyzed using latest version of Statistical Package for Social Sciences software computer program. Data for quantitative parametric data were statistically defined in terms of mean ± SD, median & interquartile range for quantitative nonparametric data, & number & percentage for qualitative data. Chai square was used to compare categorical data & Fisher's exact examination was used to compare categorical data when one or more of cell counts in 2×2 table is less than five. Independent t-test was used to compare quantitative parametric data in two independent groups and Mann-Whitney test was used to compare qualitative non-parametric data. P value greater than 0.05 was regarded as non-significant, P value equal or less than 0.05 was considered statistically important, & P value equal or less than 0.01 was considered highly statistically significant. Survival analysis was performed by estimating progression free survival (PFS), which measures how long studied cases live on cure before cancer grows, & overall survival (OS), which measures how long studied cases live after beginning therapy. Survival curves & medians for PFS & OS analysis were estimated with ninety five percent confidence using Kaplan-Meier method. Log-rank examination was used to examine relationship among survival & clinical variables.

RESULTS

Table (2) showed descriptive & comparative statistics of different studied parameters among responders & non-responders groups. Comparative statistics of demographic data among responders & non-responders groups showed highly significant statistical variation in years old among non-responders & responders groups ($t= 3.02$, $P < 0.01$). No statistically important variations were found regarding other parameters ($p > 0.05$).

Table (2): Descriptive & comparative statistics of different studied parameters between responders' & non-responders' groups.

		Responders n= 20	Non-responders n = 30	Test value	p-value	Sig.
Years old	Mean ± SD Range	54.60 ± 10.10 40 – 76	61.93 ± 7.07 48 – 76	-3.024•	0.004	HS
Sex	Females Males	5 (25.0%) 15 (75.0%)	2 (6.7%) 28 (93.3%)	3.350*	0.067	NS
Smoking	Negative Positive	5 (25.0%) 15 (75.0%)	2 (6.7%) 28 (93.3%)	3.350*	0.067	NS
Family History of Cancers	Negative Positive	17 (85.0%) 3 (15.0%)	19 (63.3%) 11 (36.7%)	2.794*	0.095	NS
Histopathology	Squamous Cell Carcinoma Adenocarcinoma Undifferentiated Large Cell Carcinoma	8 (40.0%) 11 (55.0%) 1 (5.0%)	14 (46.7%) 12 (40.0%) 4 (13.3%)	1.542*	0.463	NS
Staging	I II III IV	1 (5.0%) 3 (15.0%) 15 (75.0%) 1 (5.0%)	0 (0.0%) 2 (6.7%) 25 (83.3%) 3 (10.0%)	2.813*	0.421	NS
ECOG PS	0 1 2	9 (45.0%) 9 (45.0%) 2 (10.0%)	3 (10.0%) 14 (46.7%) 13 (43.3%)	10.577*	0.005	HS
Surgical Resection	Negative Positive	13 (65.0%) 7 (35.0%)	19 (63.3%) 11 (36.7%)	0.014*	0.904	NS
AST (IU/L)	Median (IQR)	19.5 (17.5 – 22)	22 (18 – 32)	-1.479≠	0.139	NS
ALT (IU/L)	Median (IQR)	22 (14.5 – 28.5)	18.5 (12 – 27)	-0.763≠	0.446	NS
BUN (mg/dL)	Median (IQR)	12 (8.5 – 14)	12.5 (9 – 23)	-1.312≠	0.189	NS
S.Creatinie Females (mg/dL)	Mean ± SD	0.74 ± 0.09	0.75 ± 0.07	-0.139 •	0.895	NS
S.Creatinine Males (mg/dL)	Mean ± SD	0.82 ± 0.1	1.01 ± 0.36	-1.962 •	0.057	NS

P-value > 0.05: Non significant; p-value < 0.05: Significant; p-value < 0.01: Highly significant
*: Chi-square test or Fisher's Exact test; •: Independent t-test, ≠: Mann-Whitney test

Descriptive and comparative statistics between responders and non-responders groups regarding the ERCC1 rs 11615 genotype and (T/C) allele frequencies using Chi-square test are shown in table (3). The ERCC1 rs11615 was genotyped in all studied subjects. In responders' group, 19 patients (95%) had the wild type of homozygous CC genotype and one patient (5%) had heterozygous TC genotype. In non-responders' group, 29 patients (96.7%) had the wild type of homozygous CC genotype and one patient (3.3%) had TC genotypes. TT genotype wasn't found among our studied patients (0%). There was no significant statistical variation detected among responders & non-responders groups regarding genotype frequencies (x^2= 0.8, p>0.05). Regarding allelic frequencies of ERCC1 rs 11615 polymorphism, the C allele was present in 97.5% of responders' group & 98.3% for non-responders' group. The T allele was present in 2.5% of the responders' group and in 1.7% in non-responders' group (p>0.05) (Table 3 & figure 1).

Table (3): Descriptive & comparative statistics among responders' & non-responders' groups regarding the ERCC1 rs 11615 genotype and (T/C) allele frequencies using Chi-square test

		Responders n = 20	Non-responders n = 30	X^2	p-value	Sig.
ERCC1 Genotyping	CC	19 (95.0%)	29 (96.7%)	0.087*	0.768	NS
	TC	1 (5.0%)	1 (3.3%)			
ERCC1 Gene allele	C	39 (97.5%)	59 (98.3%)	0.085*	0.771	NS
	T	1 (2.5%)	1 (1.7%)			

P-value > 0.05: Non-significant.

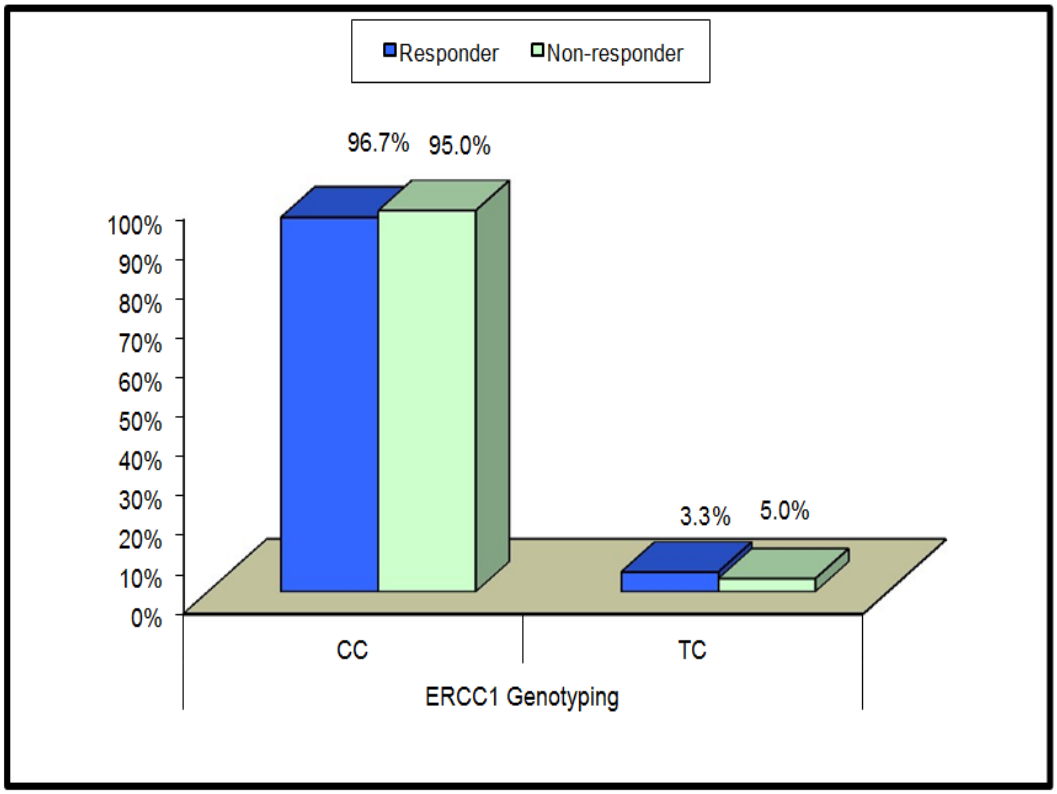

Figure (1): Bar chart showing ERCC1 genotypes frequencies in responders and non-responders.

Table (4) showed descriptive and comparative statistics between patients according to ERCC1 rs 11615 genotype in different studied parameters. Non-significant variation was showed between different ERCC1 rs 11615 genotypic polymorphism groups regarding different studied parameters (p>0.05).

Table (4): Descriptive & comparative statistics among patients according to ERCC1 rs 11615 genotype in different studied parameters

Parameter		ERCC1 rs1161 Genotype		Test value	p-value	Sig.
		CC	TC			
		n. = 48	n. = 2			
Years old	Mean ± SD Range	59.38 ± 9.06 40 – 76	50.00 ± 1.41 49 – 51	1.448•	0.154	NS
Sex	Females Males	7 (14.6%) 41 (85.4%)	0 (0.0%) 2 (100.0%)	0.339*	0.560	NS
Smoking	Negative Positive	7 (14.6%) 41 (85.4%)	0 (0.0%) 2 (100.0%)	0.339*	0.560	NS
Family History of Cancers	Negative Positive	34 (70.8%) 14 (29.2%)	2 (100.0%) 0 (0.0%)	0.810*	0.368	NS
Histopathology	Squamous Cell Carcinoma Adenocarcinoma Undifferentiated Large Cell Carcinoma	20 (41.7%) 23 (47.9%) 5 (10.4%)	2 (100.0%) 0 (0.0%) 0 (0.0%)	2.652*	0.266	NS
Staging (T,N,M)	I II III IV	1 (2.1%) 5 (10.4%) 38 (79.2%) 4 (8.3%)	0 (0.0%) 0 (0.0%) 2 (100.0%) 0 (0.0%)	0.521*	0.914	NS
ECOG PS	0 1 2	11 (22.9%) 23 (47.9%) 14 (29.2%)	1 (50.0%) 0 (0.0%) 1 (50.0%)	1.823*	0.402	NS
Surgical Resection	Negative Positive	30 (62.5%) 18 (37.5%)	2 (100.0%) 0 (0.0%)	1.172*	0.279	NS
AST(IU/L)	Median (IQR)	21 (17.5 – 25)	20.5 (19 – 22)	-0.074≠	0.941	NS
ALT(IU/L)	Median (IQR)	20 (13.5 – 28.5)	18 (12 – 24)	-0.322≠	0.747	NS
BUN(mg/dL)	Median (IQR)	12 (9 – 17)	24 (13 – 35)	-1.367≠	0.172	NS
S.Creatinine	Mean ± SD	0.90 ± 0.28	1.20 ± 0.57	-1.414•	0.164	NS

P-value > 0.05: Non significant; •: Independent t-test; ≠: Mann-Whitney test *: Chi-square test or Fisher's Exact test

The enrolled 50 non-small cell lung cancer studied cases were followed up until December 2021. Descriptive results showed that one patient had disease recurrence at end of second year. During first year of follow up 15 patients died, 2 patients from the responders and 13 patients from the non-responders. During the 2nd year of follow up 2 patients died that were from the non-responders' group. Kaplan-Meier analysis for relation of PFS (months) with ERCC1 genotyping, histopathology, years old, Eastern Cooperative Oncology Group Performance Status score, and smoking is shown in table (5). Results showed a highly significant statistical association between the ECOG-PS and PFS ($p < 0.01$) as shown in figure (2). There was a significant association among age (<60 years) & PFS ($p<0.05$) as shown in figure (3). There is an increase of PFS in Adenocarcinoma and negative smoking.

Table (5): Kaplan-Meier analysis for the Relation of PFS (months) ERCC1 rs 11615 Genotype, Histopathology, Age, ECOG PS and Smoking

		Total N	N of Events	PFS		95% CI		PFS (%)			Log Rank test		
				Mean	SE	Lower	Upper	6 m	12 m	24 m	X^2	p-value	Sig.
ERCC1 Genotype	CC	48	30	13.125	1.292	10.592	15.658	39.6%	39.6%	33.9%	0.182	0.669	NS
	TC	2	1	15.000	6.364	2.527	27.473	50.0%	50.0%	50.0%			
Histopathology	Squamous Cell Carcinoma	22	15	12.545	1.911	8.800	16.291	36.4%	36.4%	27.3%	1.973	0.373	NS
	Adenocarcinoma	23	12	14.609	1.875	10.934	18.283	47.8%	47.8%	47.8%			
	Undifferentiated Large Cell Carcinoma	5	4	6.800	0.716	5.398	8.202	20.0%	20.0%	20.0%			
Age (years)	< 60 yrs	22	8	17.455	1.846	13.836	21.073	63.6%	63.6%	63.6%	10.435	0.001	HS
	> 60 yrs	28	23	9.857	1.427	7.060	12.654	21.4%	21.4%	14.3%			
ECOG PS	0	12	3	19.500	2.250	15.090	23.910	75.0%	75.0%	75.0%	13.33	0.001	HS
	1	23	14	13.043	1.832	9.453	16.634	39.1%	39.1%	39.1%			
	2	15	14	8.400	1.640	5.187	11.613	13.3%	13.3%	0.0%			
Smoking	Negative	7	2	18.857	3.073	12.833	24.881	71.4%	71.4%	71.4%	3.612	0.057	NS
	Positive	43	29	12.279	1.331	9.669	14.889	34.9%	34.9%	29.1%			

P-value > 0.05: Non-significant; p-value < 0.05: Significant; p-value < 0.01: Highly significant S.E: Standard Error

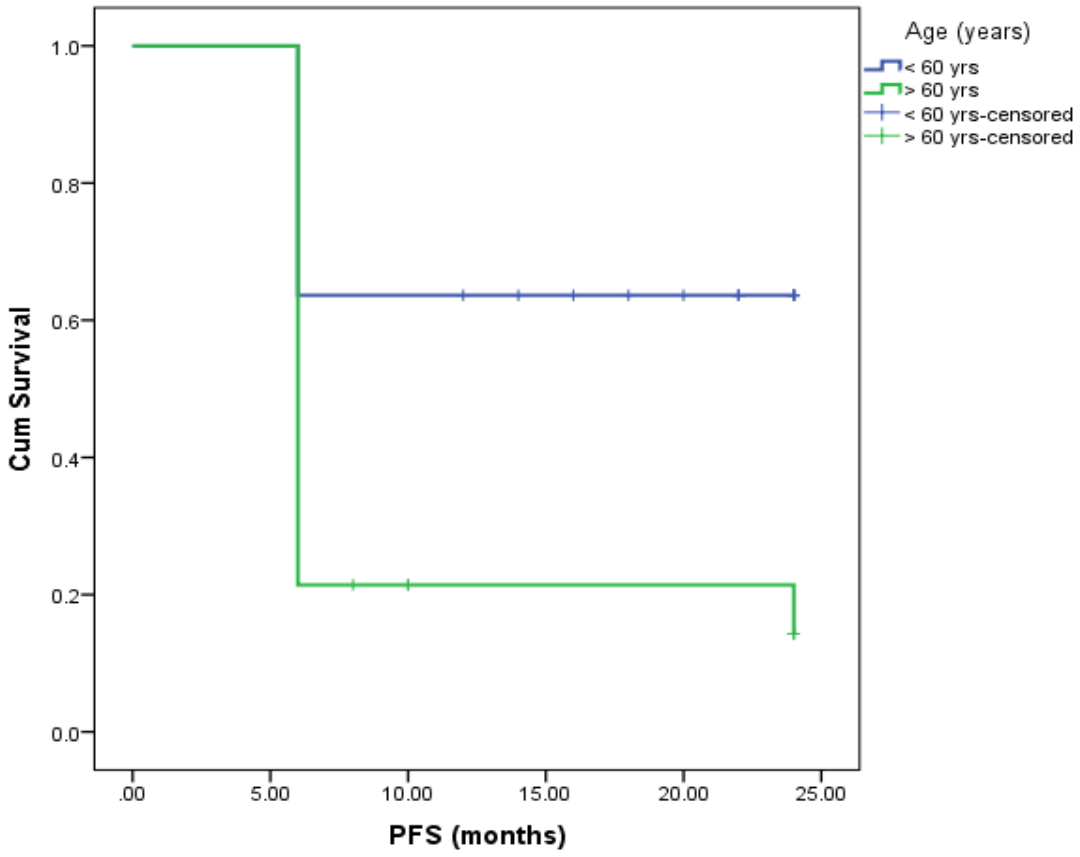

Figure (2): Kaplan Meier curve for progression free survival in relation to age

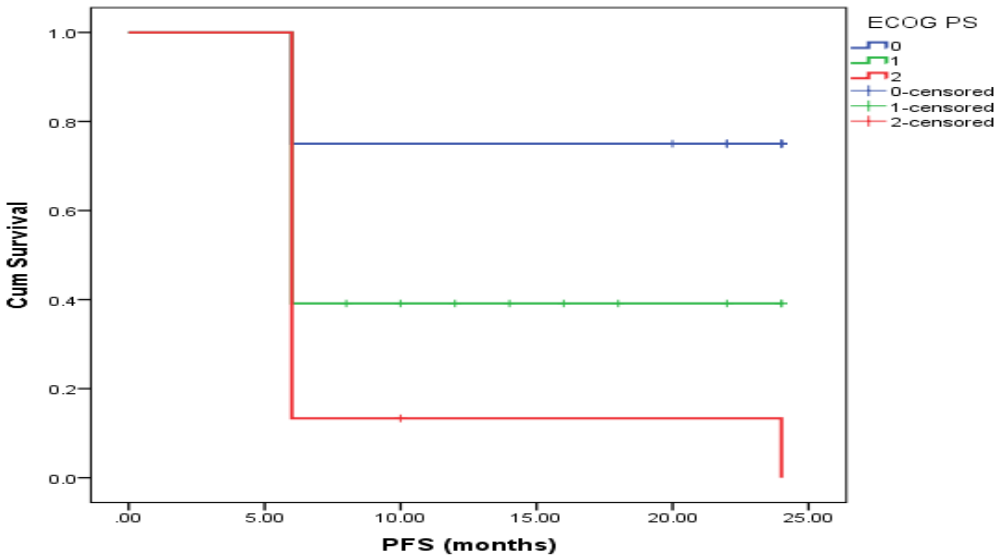

Figure (3): Kaplan Meier curve for progression free survival in relation to to Eastern Cooperative Oncology Group Performance Status.

Kaplan-Meier analysis for relation of overall survival (OS) (months) with pathology, age and ECOG PS is shown in table (6). Results showed a highly statistically significant association between age (< 60 years), ECOG PS and the OS (p <0.01) as shown in figures (4) & (5), respectively. Moreover, the OS is significantly associated between with adenocarcinoma (p<0.05) as shown in figure (6). Association among the ERCC1 genotyping & the OS could not be assessed as all the dead patients were CC genotyped.

Table (6): Kaplan-Meier analysis for the relation of OS (months) with pathology, age and ECOG (PS)

		Total N	N of Events	OS (months)		95% CI		PFS at			Log Rank test		
				Mean	S.E	Lower	Upper	6m	12m	24m	X2	P-value	Sig.
Histopathology	Squamous Cell Carcinoma	22	8	17.66	1.78	14.17	21.15	86.4	62.4	62.4	7.413	0.025	S
	Adenocarcinoma	23	7	23.26	0.35	22.58	23.95	100	100	59.2			
	Undifferentiated Large Cell Carcinoma	5	2	11.60	1.43	8.80	14.41	80	60	60			
Age	< 60 years	22	2	23.01	0.81	21.424	24.593	95.5	95.5	86.8	11.532	0.001	HS
	> 60 years	28	15	16.32	1.35	13.686	18.958	96.4	45.9	40.2			
ECOG	0	12	0	–	–	–	–	–	–	–	7.762	0.005	HS
	1	23	6	20.36	1.31	17.781	22.93	95.7	76.9	68.4			
	2	15	11	14.12	1.68	10.836	17.405	93.3	30.5	20.3			

P-value > 0.05: Non significant; p-value < 0.05: Significant; p-value < 0.01: Highly significant S.E: standard error

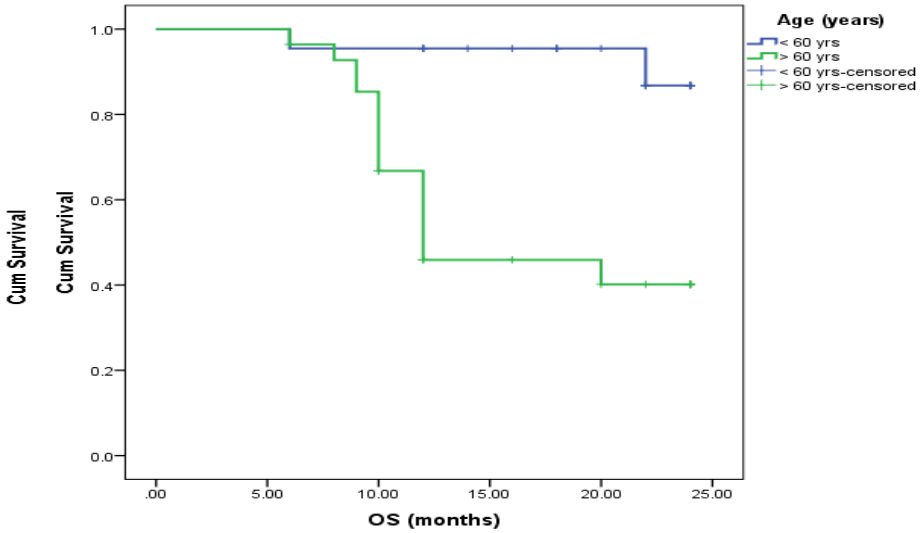

Figure (4): Kaplan Meier curve for overall survival (OS) in relation to age

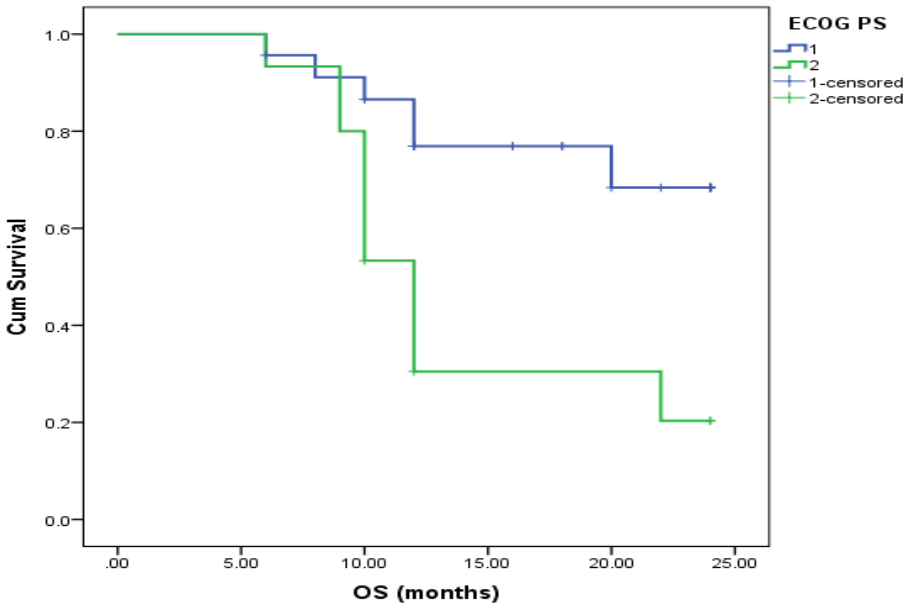

Figure (5): Kaplan Meier curve for overall survival (OS) in relation to Eastern Cooperative Oncology Group Performance Status

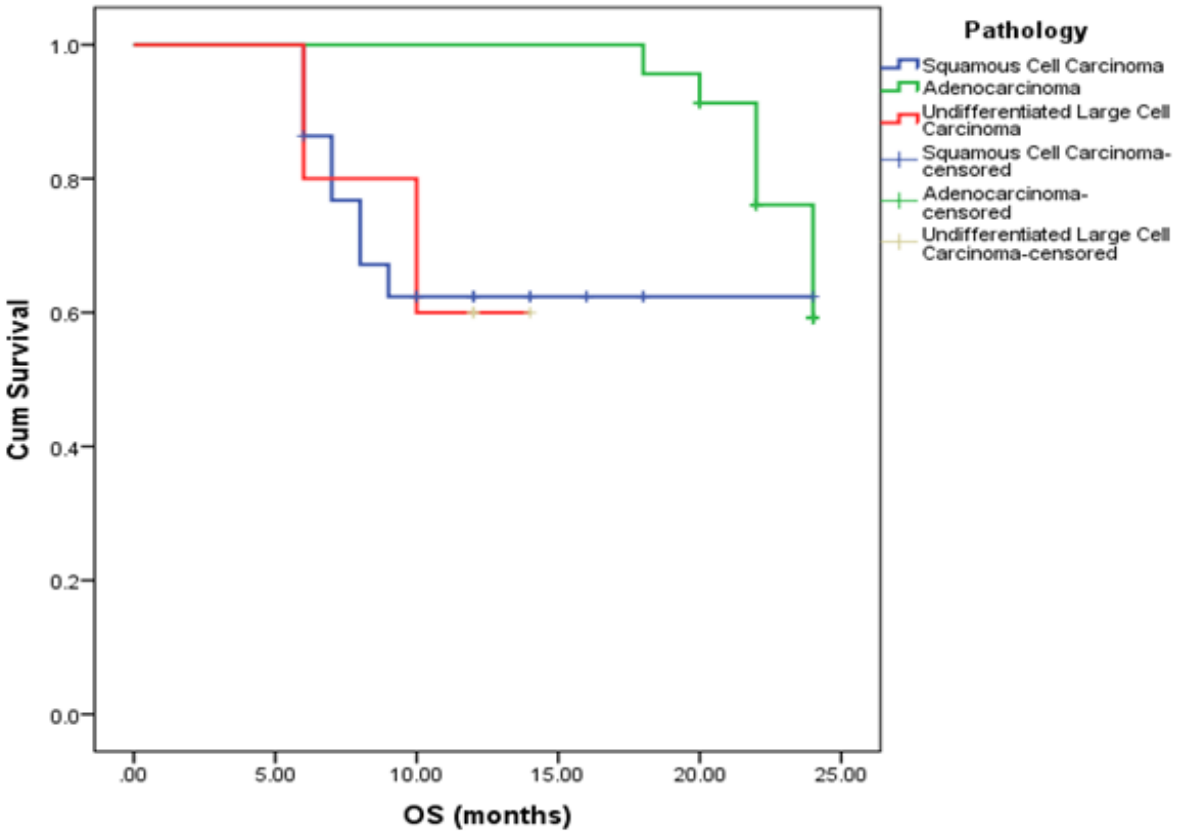

Figure (6): Kaplan Meier curve for overall survival in relation to pathology.

DISCUSSION

Lung cancer (LC) is severe & rapidly spreading public health issue. It is important reason for cancer-related death worldwide, with five-year survival rate ranging from five to ten years [9]. According to the histopathological features, non-small cell lung cancer is major type [10]. Although LC has generally poor prognosis, early-stage LC has better prognosis & is more amenable to cure [11].

Platinum-based anticancer drugs are main chemotherapeutic cure of lung cancer [7]. They act on DNA in nucleus, inhibiting DNA replication & thus exerting cytotoxic effects [12]. Their clinical effectiveness is limited by toxicity and the potential for the targeted cells to develop resistance [13]. When platinum interacts with the nuclear DNA, intra-strand adducts & intra- & inter-strand crosslinks are formed and this will lead to activation of DNA repair pathways, initially nucleotide excision repair pathway & mismatch repair pathway, &

activation of DNA damage response through ataxia-telangiectasia mutated & Rad3-related activation & p fifty three activation. This can cause cell cycle arrest at S, G1 & G2-M phase & activation of apoptosis [14]. Nucleotide excision repair system is crucial in platinum resistance to chemotherapy. It heals platinum-induced DNA damage by removing damaged DNA fragments. ERCC1 gene is important in NER [15].

Some clinical researches have suggested link among ERCC1 mRNA levels & cisplatin drug resistance **[16]**. This implies that looking at ERCC1 expression levels could be good way to recognize studied cases who will benefit from cisplatin-based chemotherapy. Previous research has linked ERCC1 rs 11615 polymorphism to cure response in variety of cancers, including gastric, esophageal, & colorectal cancers [17].

We conducted our study on 50 NSCLC studied cases who were recruited from Oncology Clinic in Ain Shams University Hospitals; twenty of them showed response to platinum-based chemotherapy & other thirty were not responders according to RECIST criteria.

There was statistically significant variation in age among responders & non-responders being higher in non-responders than responders. Our findings come in agreement with those conducted by ***Tas et al.*** [18] who discovered that elderly studied cases had worse results than younger studied cases. Lung cancer is common in elderly [19]. Due to obvious progressive loss of organ function, modifications in functional status, drug pharmacokinetics, & age-related comorbidities. Elderly studied cases tolerate chemotherapy poorly compared to their younger counterparts [20-22]. Moreover, Concomitant diseases, which can have significant impact on functional status, general health, & tumour symptoms, are common in this studied case population [23]. On contrary, case-control study by ***Costa et al.*** **[24]** suggested that studied cases over seventy years old with NSCLC stage III & IV getting platinum-based treatment had better 2- & 3-year survival rates than younger studied cases matched for cancer stage & year of cure. Although, these outcomes are intriguing, Costa & his colleagues explained that elderly chemotherapy studied cases may have been chosen to be relatively healthier than their younger counterparts due to physician selection & studied case preference. Explanation of the better response to platinum-based chemotherapy among elderly may be attributed to the less effectiveness of NER pathway in old age [24].

Our studied NSCLC patients were genotyped for the detection of ERCC1 rs11615 polymorphism. The wild CC genotype was the predominant one representing 96%, while TC genotype represented 4% and unexpectedly, the TT genotype could not be detected. According to our studied groups, the TC genotype represented only 5% and 3.3% in responders and non-responders groups, respectively. These findings explain the absence of statistically significant variation in genotype frequencies when comparing the responders and non-responders groups. Our data are in agreement with those of ***Zhou et al., Ludovini et al.,*** & ***Kalikaki et al.*** who found no significant association among ERCC1 rs 11615 genotypes & objective response [25-27]. ***Zhou* and co-workers** **[25]** performed their study on Chinese patients in which 64.4% had CC genotype while 35.4% had TC and TT genotypes. The Italian literature by ***Ludovini* and colleagues** **[26]** found that the frequencies of the ERCC1 genotypes (CC) and (TC and TT) were 28.6% and 71.4% respectively. The Greek ***Kalikaki* and colleagues** **[27]** found that CC and TC genotypes represented (78%) and TT (22%) in their studied sample. We suggest the role of Ethnicity in genotype frequency regarding the ERCC1 gene; standing on the previous data and on the fact that all included studied cases in research were different ethnicity [25-27].

The T allele in responders' group (2.5%) slightly exceeded the non-responders' group (1.7%). However, our data revealed absence of a statistically significant difference in the allelic frequencies regarding response. Previous data by ***Zhou et al.*** **[25]**, ***Ludovini et al.*** **[26]**, & ***Kalikaki et al.*** **[27]** with more frequent T allele showed non-significant effect on platinum response. On the contrary, the meta-analysis of 12 researches conducted by ***Wei et al.*** **[28]** in China showed that platinum-based chemotherapy sensitivity was significantly related to polymorphism of ERCC1 rs 11615. On the other hand, a poor response to platinum was reported in case control study conducted by ***Gao et al.*** **[29]** on 163 patients. This relative larger sample size might have influenced the result. A Chinese study by ***Zhao et al.*** [30] & Spanish study by ***Sullivan et al.*** [31] reported that ERCC1 rs 11615 studied cases with at least one T allele (TT or TC) showed greater response rate than those with a CC genotype. On other hand, meta-analysis performed by ***Wei et al.*** [28] demonstrated that studied cases with NSCLC with CC/CT genotypes in ERCC1 rs 11615 polymorphism exhibited greater chance of response to platinum-based cure than those with TT genotype. ***Gao et al.*** [29] also found that individuals carrying rs11615 TT genotype & T allele found significantly lower response rate to chemotherapy using rs11615 CC genotype like reference. In 2013, ***Xu*** and his colleagues performed large meta-analysis based on 39 reports to determine the association of ERCC1 -C118T- & -C8092A polymorphisms with lung cancer & survival of advanced-stage NSCLC studied cases getting platinum-based chemotherapy. They found no association among ERCC1 C118T & response to cure [32].

Our findings may be because of complexity of the DNA repair systems as they act together, or there are some other mutations, which affect the repair pathways or presence of other gene polymorphisms, which interfere with cytotoxic drugs of mixture treatment; precluding the prediction of responses based on ERCC1 in NSCLC studied cases. A network meta-analysis of twenty six cohort researches was conducted by ***Yu et al.***

[33] to compare predictive value of fourteen SNPs in different DNA repair genes on the efficacy of platinum based-chemotherapy in NSCLC studied cases. They concluded that ERCC1 (rs 11615), XRCC1 (rs2548, rs179982) and XPD (rs 131181) are better predictors of platinum-based chemotherapy efficacy in NSCLC studied cases [33].

We assessed survival rates of our studied patients in time period from May 2019 to December 2021. Studied cases with younger years old (<60 years), ECOG PS zero & adenocarcinoma significantly showed better survival; progression free survival & overall survival. Association among ERCC1 rs 11615 genotype and the OS could not be assessed as all the dead patients were carrying CC genotype. Despite we found no statistical association among ERCC1 genotypes & the PFS, we noticed that patient carrying TC genotype had longer PFS than others, which suggest that T allele may have an impact on the disease survival and treatment response. Our findings are supported by ***Kalikaki et al.*** [27] who found that among 5 examined polymorphisms, ERCC1- C8092A was only 1, which was related to PFS. Combined analysis of 2 ERCC1 polymorphism found that number of polymorphic variants of ERCC1 is significantly related to PFS [27]. On the other hand, ***Ryu et al.*** [34] showed that NSCLC receiving platinum-based chemotherapy who had ERCC1 rs 11615 CC had apparently longer survival & time to progression than those harboring non-wild type ERCC1. Further research by ***Zhao et al.*** [30] found that ERCC1 rs 11615 was related to poor response to chemotherapy & shorter survival time of progressive NSCLC.

ERCC1 rs 11615 polymorphism may affect response to platinum chemotherapy & survival as it affects DNA repair system, which is main target of platinum. However, neither the homozygous of ERCC1 rs 11615 CC nor the heterozygous TC showed an effect on treatment response. This can be attributed to small sample size. Larger sample researches are needed to approve our findings.

CONCLUSION

In conclusion, our researchers reported no evidence of link among ERCC1 rs 11615 polymorphism & response to platinum-based chemotherapy in progressive cases of NSCLC. These data set may provide useful platform for research & clinical health practice in identifying molecular biomarkers that may impact reaction to platinum-based chemotherapy treatment & selecting those studied cases most likely to gain from cost & toxicity avoidance.

AUTHORS' CONTRIBUTION

HAZ was in charge of conceptualization as well as supervising, reviewing, & validating final manuscript. MMM & DAM both contributed to manuscript's writing & editing, as well as to research methodology, while AIA assisted with manuscript review & sample collection. REM was in charge of original draught preparation & writing, as well as sample collection & research methodology. Final manuscript was read, revised, & approved by all authors.

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