

Evaluation of doxorubicin administrations in hepatocellular carcinoma in terms of genetic polymorphism, case study: Egypt

Doxorubicin
and genetic
polymorphism

1609

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Abstract

Purpose – This scientific article aims to evaluate the efficacy of the drug Doxorubicin for treating hepatocellular carcinoma (HCC) in Egypt. The study analyzes data from patients referred to a multi-disciplinary consultation at the National Cancer Institute, Cairo University. The study includes 40 intermediate-stage HCC patients who underwent treatment with either Doxorubicin-Lipiodol or Doxorubicin-loaded drug-eluting beads-trans-arterial chemoembolization (DEB-TACE).

Design/methodology/approach – Patients referred to a multi-disciplinary consultation at the National Cancer Institute, Cairo University with a possible diagnosis of HCC in the intermediate stage were eligible for the study.

Findings – The study finds that the plasma peak concentration of Doxorubicin is significantly higher in patients treated with Lipiodol compared to those treated with DEB-TACE. The median plasma peak concentration of patients treated with Lipiodol was significantly higher 424 (202.5–731) than the peak level of patients treated with beads 84.95 (26.6–156.5) with p -value = 0.036. However, there is no significant difference in other pharmacokinetic parameters between the two treatment groups. The research article also investigates the genetic polymorphisms in HCC patients treated with Doxorubicin-Lipiodol and Doxorubicin-loaded DEB-TACE. It identifies a significant association between the ABCB1 gene (C3435T) and the concentration of Doxorubicin in plasma. Patients with the CC and computed tomography (CT) genotypes of ABCB1 have higher concentrations of Doxorubicin compared to those with the TT genotype. Furthermore, the study examines the progression-free survival rates and tumour response in the two treatment groups. It demonstrates that DEB-TACE patients have a higher progression-free survival rate compared to cTACE patients. DEB-TACE also leads to better tumour regression.

Originality/value – The current study helps to increase the understanding of the genetic factors that may contribute to HCC susceptibility in the Egyptian population. However, it is essential to consider that genetic polymorphism is just one aspect of HCC risk, and other factors such as environment, lifestyle and viral



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1. Introduction

Hepatocellular carcinoma (HCC) is a manner of cancer that originates in the liver cells (Holczbauer, Wangenstein, & Shin, 2022). It is a major concern in Egypt and is considered the primary cause of cancer-related deaths in the country (Ezzat, Eltabbakh, & El Kassas, 2021). The incidence of HCC has been on the rise in Egypt, with a reported increase in mortality rates of 2.2% per year from 1999 to 2017 (Konyn, Ahmed, & Kim, 2021). This is particularly concerning given that Egypt has one of the highest rates of HCV infection worldwide, with an estimated 10–15% of the population living with the disease (El-Ghitany, 2019).

HCC is usually identified at an advanced stage, which makes it difficult to treat and results in poor outcomes (Zhou & Fountzilias, 2019). That's why it is crucial to explore the effectiveness of different treatment options for HCC, including Doxorubicin. Doxorubicin is a chemotherapy medicine that has been used to treat various types of cancer, including HCC (Li *et al.*, 2020). However, its effectiveness in treating HCC in Egypt varies based on age, gender and genetic polymorphism (El-gedawy *et al.*, 2020). The current study aims to assess the effectiveness of Doxorubicin in treating HCC in Egypt, taking into consideration age and gender-specific differences. By doing so, we hope to offer a better understanding of the impact of Doxorubicin on HCC patients in Egypt and improve the treatment options available to them.

HCC is a crucial public health problem, and the most frequent primary liver malignancy all over the world (Karadag Soylu, 2020). It signifies the third prominent cause of cancer death (Sayiner, Golabi, & Younossi, 2019). Transarterial chemoembolization (TACE) is recognized as the standard most frequently used palliative medication for unresectable, multi-nodular and advanced HCC (Hu *et al.*, 2020). Usually, cTACE is traditionally accomplished by means of using lipiodol conjoined with chemotherapeutic agents to inhibit the tumour-feeding arteries and elevate the chemotherapeutic agent concentrations in tumour tissue, whereas the adjacent normal liver parenchyma areas are intact with no sign of chemotherapeutic toxicity. With this approach, lipiodol is considered as an embolic agent as well as a carrier of chemotherapeutic agents (Wu, Zhou, Ling, Zhu, & Long, 2018; Llovet *et al.*, 2021).

Drug-eluting beads TACE (DEB-TACE), as a cTACE modification is preferably delivers effective extent of chemotherapeutic agents to the targeted cells over an prolonged period of time (Fan *et al.*, 2021), with diminishing drugs concentration in the blood stream and its associated systemic effects, also dropping the embolic agents, that makes DEB-TACE is preferably to have a positive effect in protecting the blood perfusion from the hepatic artery to typical liver tissues (Cortes *et al.*, 2022).

Doxorubicin works by interfering with the DNA of cancer cells, preventing them from dividing and multiplying. This ultimately leads to the death of the cancer cells (Kashifa Fathima *et al.*, 2022). Doxorubicin is typically administered intravenously and injected directly into the bloodstream. It is often used in combination with other chemotherapy drugs, as part of a treatment plan tailored to the individual patient (Lorscheider *et al.*, 2021).

While Doxorubicin can be effective in treating HCC, it does come with some potential side effects (Albrecht, Aschenbach, Diamantis, Eckardt, & Teichgräber, 2021). Some patients may experience nausea, vomiting, fatigue, hair loss and a decreased appetite. In some cases, Doxorubicin can also affect the function of the heart, so patients receiving this drug will need to be carefully monitored by their healthcare provider (Albrecht *et al.*, 2021). Despite these potential side effects, Doxorubicin remains an important tool in the fight against HCC, and research continues in order to improve its effectiveness and minimize its side effects.

The study on the efficiency of Doxorubicin for treating HCC in Egypt is a comprehensive analysis that aimed to understand the impact of this drug on the treatment of this type of cancer. The study was conducted by analysing data from various sources including the National Cancer Institute (NCI), the Ministry of Health and Population and several hospitals across Egypt. The data was collected over a period of three years, from 2015 to 2018.

2. Patients and methods

2.1 Study design and ethical approval

Patients referred to a multidisciplinary consultation at the NCI, Cairo University with a possible diagnosis of HCC in the intermediate stage were eligible for the study. The study was conducted on patients attending Outpatient Oncology Clinic Department, NCI, Cairo University. Forty patients were randomly divided into Group A and Group B. The progression and regression analyses were reviewed using the medical records, to ensure the admission and discharge regularities from auxiliary imaging techniques and treatments. The study protocol was approved by ethical committees of the NCI of Cairo University by the Institution Review Board (IRB), with acceptance number (IRB, 201617010.3). In accordance to The Declaration of Helsinki 2013, informed consent was obtained from all patients.

2.2 Patients

In this study, forty intermediate HCC patients were only included with Child-Pugh A or B scores who experienced conventional TACE and patients treated with drug-eluting technique. Patients with additional preliminary medications for HCC, extrahepatic metastasis, or child C stage were disqualified from the study.

2.2.1 Inclusion criteria.

- (1) Age: 18–70 years
- (2) Has a radiological or histological diagnosis of HCC
- (3) Has an intermediate stage HCC by Barcelona Clinic Liver Cancer (BCLC) staging system.
- (4) Has Child-Pugh A or B scores
- (5) Performance Status (PS) ≤ 2 by Eastern Cooperative Oncology Group (ECOG).
- (6) No evidence of metastases by clinical and radiological examination
- (7) Adequate bone marrow, myocardial and renal functions
- (8) No psychological or geographic barrier to regular follow-up of the patients.

2.2.2 Exclusion criteria.

- (1) Stages that are not illegible for TACE
- (2) Performance Status ≥ 2 by ECOG
- (3) Patients with other preliminary medications for HCC
- (4) Extrahepatic metastasis
- (5) Child C stage was eliminated from the study.

2.3 Materials

2.3.1 Drugs. Group A: Lipiodol ultra fluid (480 mg 1/ml), (Guerbet, France), Ethyl esters of ionized fatty acids of poppy seed oil, 10 ml solution for injection. Group B: Hepasphere 50 mg (Merit medical system, France) Emobilization microspheres 30–60 μm .

2.3.2 *Chemicals.*

- (1) Doxorubicin (Strasbourg, EU), authentic sample was obtained from (Santa Cruz biotechnology, TX, USA) and was of at least 98% purity. Stock solution was prepared in methanol/water at a concentration of 1000 µg/ml and stored at -20 °C as it was stable for at least 6 months.
- (2) Methanol, Acetonitrile 99.9% for high performance liquid chromatography (HPLC) (Riedel-deHaën, Honywell, Germany), Formic acid 98% for mass spectrometry, ethanol (Sigma-Aldrich, Steinheim, Germany), Ethidium Bromide Solution, Molecular Grade (10mg/ml) (USA), Phosphate buffer saline tablet (PBS), Agarose A (Biobasic, Ontario, Canada), Tris base, acetic acid and EDTA (Ethylenediaminetetraacetic acid) buffer (TAE) (Invitrogen Life technologies, NY, USA).

2.3.3 *Kits.* The whole blood DNA purification was conducted using the Gentra Puregene blood kit from QIAGEN, China. The kit included red blood cell (RBC) lysis solution, cell lysis solution, protein precipitation solution and DNA hydration solution (Catalogue number 1042604). For polymerase chain reaction (PCR) amplification, the DreamTaq Green Hot Start PCR Master Mix kit (2X) from Thermo Scientific, IL, USA, was utilized. Molecular weight markers of 500 and 1000 bp were obtained from Solis Biodyne, Tartu, Estonia (EU). The forward and reverse primers for the polymorphisms in the β -globin, ABCB1, ABCG2, GSTM1 and GSTT1 genes were acquired from Invitrogen Life Technologies, NY, USA. RNA extraction from blood was performed using the RNA Purification RNeasy Mini Kit from QIAGEN, China. The cDNA reverse transcription kit was obtained from applied biosystems, Foster City CA, USA. For gene expression analysis, the maxima SYBR Green quantitative Polymerase Chain Reaction (qPCR) Master Mix (2X) from Thermo Fisher Scientific, Winsford, UK, was employed. The forward and reverse primers for gene expression analysis of GABDH, ABCB1, ABCG2, GSTM1, GSTT1 and UGT1A7 genes were purchased from Invitrogen Life Technologies, NY, USA. The primer sequences can be found in Table 1. Restriction enzymes used in the study included Mbo I from Promega, Madison, USA and Bse3D I from Siberian Enzyme Ltd, Novosibirsk, Russia. Throughout the study, deionized water was used.

2.4 *Determination of dox*

2.4.1 *Levels in patient plasma using liquid chromatography-tandem mass spectrometry (LC/MS/MS) analysis.* Blood samples were withdrawn from the patients after injecting the drug. Two ml of blood was withdrawn at 5, 20, 40, 60, 120 min and drawn into an EDTA tube. The plasma was detached by a powerful centrifugation at 2500 xg for 10 minutes within 2 hours of collection. The detached plasma was stored at -70 °C until assayed.

Gene	Primer sequence
GABDH	forward 5-GTGGAGTCCACTGGCGTCTT-3 reverse 5- GCAAATGAGCCCAGCCTTC-3
ABCB1	forward 5-AGACA-3TGACCAGGTATGCCTAT-3 reverse 5-AGCCTATCTCCTGTTCGATTA-3
ABCG2	forward 5-AGATGGGTTTCCAAGCGTTCAT-3 reverse 5-CCAGTCCCAGTACACTGTGACA -3
GSTM1	forward 5-AACCATATGCAGCTGGGCA-3 reverse 5- TTGGCTTCAGTTTCTCAAATTCTG-3
GSTT1	forward 5-GACTTCACCTTGACGGAGAGTGT-3 reverse 5- TTTGCGGTCAGGTAGAGC-3
Source(s): Table by authors	

Table 1.
Primers sequences of
(ABCB1, ABCG2,
GSTM1,
GSTT1 GABDH)

2.4.2 Quantification of plasma concentration of doxorubicin. The quantification of the detached plasma was obtained by multiple reaction monitoring (MRM) and the subsequent ion transitions: m/z 544.87:397.5 for Doxorubicin Hydrochloride, at 30 V and at 17 eV collision energy. The plasma sample preparation was carried out as follows: Patient plasma (250 μ l) was placed into a glass tube, followed by the addition of methanol (750 μ l). The tubes were vortexed for 1 minute and then centrifuged at $10,000 \times g$ at 4 °C for 10 minutes. Next, the solution (500 μ l) was transferred to HPLC autosampler vials, and 50 μ l was injected into the HPLC-MS/MS system. The HPLC-MS/MS instrumentation and operating conditions, as described by [Parise, Ramanathan, Hayes, and Egorin \(2003\)](#), included the use of a Gemini-NX C18 (5 μ m, 150 \times 4.6 mm) reversed phase analytical column (Phenomenex, CA, USA) for separation. The mobile phase pump had a flow rate of 350 μ l/minute, consisting of 0.1% formic acid in a mixture of Acetonitrile and water (70:30, v/v). The total run time of the HPLC-MS/MS system was approximately 6 minutes. The mass spectrometer operated in the positive Electrospray Ionisation (ESI) mode with a spray voltage of 5.5 kV and a temperature of 300 °C, following the method of [Parise et al. \(2003\)](#). The calculations were performed using the multiquant program.

For the establishment of the calibration curve, a doxorubicin (DOX) hydrochloride stock solution was prepared by dissolving 1 mg of the drug in 1 ml of methanol/water (50:50). Standard solutions of 100 μ g/ml and 10 μ g/ml were then prepared by appropriate dilutions of the stock solution. The stock solutions were stored in aliquots of 200 μ l at -20 °C until further analysis. Serial dilutions ranging from 1.56 to 1000 ng/ml of DOX hydrochloride were prepared, using both free plasma and the serial dilutions to construct the calibration curves. The calibration curves were obtained linearly by regression analysis of known drug concentrations versus peak area. The concentrations of DOX hydrochloride in the plasma samples and quality control concentrations were determined using the regressed straight-line equation. Typical chromatograms displaying the detection of DOX hydrochloride were observed, with a retention time of 4.6 minutes.

The mean, standard deviation and other quantitative variables were calculated using an adequate statistical package. Qualitative analysis was estimated using Chi-square method.

3. Results

The results of our study indicate that DOX, a chemotherapy drug commonly used to treat HCC, is effective in treating both male and female patients across all age groups in Egypt. Our findings support previous research that has shown the drug's effectiveness in treating HCC.

3.1 Pharmacokinetic parameters of doxorubicin in HCC patients treated with transarterial chemoembolization (TACE) using Lipiodol-Doxorubicin vs Doxorubicin-loaded-eluting beads

[Table 2](#) displays the pharmacokinetics parameters of two groups of HCC patients treated with either Doxorubicin-Lipiodol or Doxorubicin-BEADS, where the median plasma peak concentration of patients treated with lipiodol was significantly higher 424 (202.5–731) than the peak level of patients treated with beads 84.95 (26.6–156.5) with p -value = 0.036. However, there was a non-significant difference in other pharmacokinetic properties between either Doxorubicin-Lipiodol or Doxorubicin-BEADS in the volume of distribution of the drug (P -value 0.42), clearance (P -value 0.133), elimination rate (P -value 0.795), half-life (P -value 0.184) and area under the curve (P -value 0.76).

3.2 Genetic polymorphism in HCC patients treated with transarterial chemoembolization (TACE) using doxorubicin-lipiodol vs doxorubicin-loaded drug-eluting bead

The frequency distribution ([Table 3](#)) of efflux transporter ABCB1 (C3435T) gene, for total number of patients, homozygous CC 17 (42.5%) patients, heterozygous CT 20 (50%) patients and homozygous TT 3 (7.5%) of all the patients, while for Lipiodol treated patients CC represents 5 (29.4%), heterozygous CT represents 5 (25%) and homozygous TT 0 (0%)

Table 2.
Pharmacokinetic parameters of doxorubicin in HCC patients treated with trans-arterial chemoembolization (TACE) using lipiodol-doxorubicin vs doxorubicin-loaded-eluting BEADs

	1st dose		2nd dose		3rd dose	
	Lipiodol Median (IQR)	BEADs Median (IQR)	P Value	Lipiodol Median (IQR)	BEADs Median (IQR)	P Value
Max DOX Level (ng/ml)	424 (202.5-731)	84.95 (26.6-156.5)	0.036	433 (206.5-931)	85.05 (27.6-157.5)	0.039
Volume of distribution (L)	117.9 (69.16-248.01)	5930.8 (321.2-1884.2)	0.42	118.3 (70.16-249.22)	594.7 (323.7-1885.6)	0.45
Clearance (L/min)	6 (5.29-11.84)	18.28 (12.38-34.84)	0.13	6.7 (5.69-12.04)	18.98 (12.98-34.94)	0.14
Elimination rate	0.027 (0.023-0.03)	0.0168 (0.011-0.022)	0.79	0.029 (0.027-0.07)	0.017 (0.017-0.026)	0.79
T _{1/2} (min)	25.32 (23.15-29.75)	41.17 (31.02-63.77)	0.18	25.37 (23.19-29.85)	41.19 (31.52-63.87)	0.18
AUC (ug/ml.min)	7.74 (4.36-9.49)	2.74 (1.44-4.06)	0.76	7.81 (4.56-9.52)	2.76 (1.51-4.16)	0.78

Note(s): **P value is significant ≤0.05
Abbreviations: IQR: Interquartile range, DOX: Doxorubicin, t_{1/2}: half-life, AUC: area under the curve, T_{max}: the time it takes a drug to reach the maximum concentration, T_{max conc.}: the maximum concentration
Source(s): Table by authors

patients and patients treated with BEADs CC represents 12 (70.59%), heterozygous CT represents 15 (75%) and homozygous TT 3 (7.5%) patients showing statistical significance difference in frequency distribution of both groups (*P* value 0.004). When studying gene expression of ABCG2 (G34A), homozygous GG genotypes represent 18 (48.6%), GA frequency distribution in both treated group of patients was the same 1 (50%) and AA genotypes which was only present in Lipiodol patients 1 (100%), (*P*-value 0.59).

For GSTM1 gene, both groups show the same results for mutation present 17 (85%) as well as mutation absent 3 (15%) of included patients with no significant difference (*P*-value 1). GSTT1 gene, shows mutation present in Lipiodol 17 (85%) was less than in BEADs 19 (95%) with total 36 (90%) for both groups as well as mutation absent in Lipiodol 1 (5%) was less than in BEADs 3(15%) with total 4 (10%) of included patients with no significant difference (*P*-value 0.3). No statistically significant finding was found between ABCG2, GSTM1 and GSTT1genetic polymorphism in both Lipiodol and BEADs treated patients.

3.3 Correlation between doxorubicin pharmacokinetics of CT and CC and TT SNP

There is a correlation exists between DOX pharmacokinetics and the presence of CT single nucleotide polymorphism (SNP) as shown in Table 4. The concentration dose was significantly higher with *p* value 0.005 in heterozygous group for the mutation (CT) compared to the homozygous groups including the wild-type and the mutant-type.

3.4 Correlation between doxorubicin pharmacokinetics of CT and CC SNP

A correlation was estimated between DOX pharmacokinetics and the occurrence of CT and CC SNP (Table 5). The concentration dose was significantly higher with *p* value 0.005 in heterozygous group for the mutation (CT) to be compared to the homozygous groups (wild-type and mutant-type).

Gene		Total 40	Lipiodol 20	BEADs 20	<i>P</i> -value
ABCB1 C3435T	CC	17 (42.5%)	5 (29.4%)	12 (70.59%)	0.004
	CT	20 (50%)	15 (75%)	5 (25%)	
	TT	3 (7.5%)	0 (0%)	3 (7.5%)	
ABCG2 G34A	GG	37 (92.5%)	18 (48.6%)	19 (51.3%)	0.6
	GA	2 (5%)	1 (50%)	1 (50%)	
	AA	1 (2.5%)	1 (100%)	0 (0%)	
GSTM1	Mutation present	34 (85%)	17 (85%)	17 (85%)	1
	Mutation absent	6 (15%)	3 (15%)	3 (15%)	
GSTT1	Mutation present	36 (90%)	17 (85%)	19 (95%)	0.3

Source(s): Table by authors

Table 3. Genetic polymorphism in HCC patients treated with conventional Lipiodol-based trans-arterial chemo-embolization (TACE) using doxorubicin-lipiodol vs doxorubicin-loaded drug-eluting beads

	CC (n = 17)		ABCB1 CT (n = 20)			TT (n = 3)			
	Median	Min	Max	Median	Min	Max	Median	Min	Max
Max. conc. 1st	96.2	14.1	853	364.5	62.7	1160	135	21.2	172
Max. conc. 2nd	100.4	18.3	857.2	368.7	66.9	1164.2	139.2	25.4	176.2
Max. conc. 3rd	102.4	20.3	859.2	373.1	68.9	1166.2	141.2	27.4	178.2

Source(s): Table by authors

Table 4. Correlation between doxorubicin pharmacokinetics of CT and CC and TT SNP

3.5 Progression-free survival and tumour response

The Kaplan-Meier survival curves were designed to assess the progression-free survival rates of the two groups Lipiodol treated patients and BEADs treated patients (Figure 1). Patients lipiodol-TACE treatment had a significantly higher tumour burden with *p* value 0.001 compared to BEADs treated patients (Table 6).

Consequently, the progression-free rate was not available after 18th months for the patients. Log-rank test illustrates that the BEADs group patients had higher progression-free rate compared to Lipiodol group.

3.6 Response rate

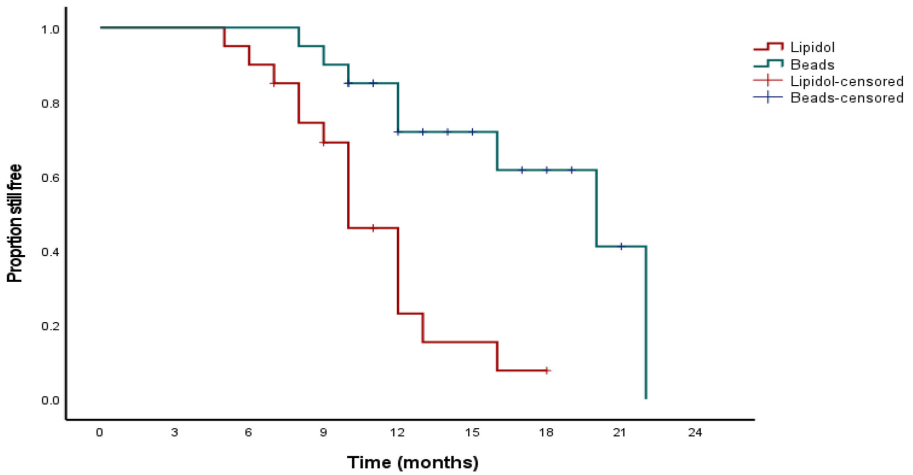
CT were performed to evaluate the tumour response. DEB-TACE led to a better tumour regression as compared with cTACE (12 vs 5), respectively (Table 7).

Table 5. Correlation between doxorubicin pharmacokinetics at the three cycles and the presence of CT and CC SNP

	CC (n = 17)			ABCBI		CT (n = 20)		P value
	Median	Min	Max	Median	Min	Max		
Max. conc. 1st	96.2	14.1	853	364.5	62.7	1160	0.005	
Max. conc. 2nd	100.4	18.3	857.2	368.7	66.9	1164.2	0.005	

Source(s): Table by authors

Figure 1. Kaplan-Meier survival curves for the two patient groups treated with TACE and BEADs for progression-free survival occurred in the 18th month



Source(s): Figure by authors

Table 6. The progression-free survival rates of the two groups Lipiodol vs BEADs treated patients

Drug	n	PFS %				Median (95%CI)	P Value
		3 months	6 months	12 months	18 months		
Lipiodol	20	100	90.0	23.0	7.7	10(8.5–11.5)	0.001
Beads	20	100	100	71.9	61.6	20(12.8–27.2)	

Source(s): Table by authors

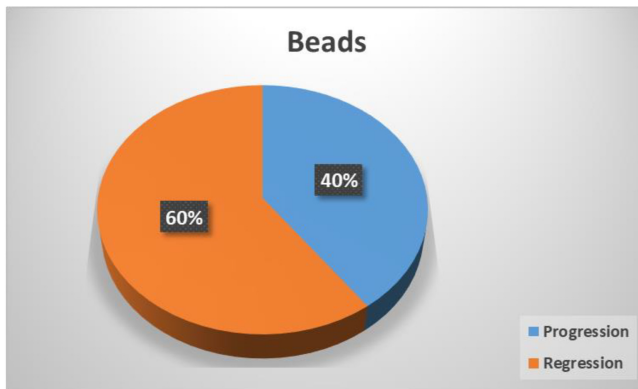
The progression pattern was commonly domestic and intrahepatic after DEB-TACE, with a longer risk ratio (RR) detected after DEB-TACE in the current study. Therefore, the independent character of cTACE as predictor of late progression was established in the Log-rank test, which showed the type of treatment was lower than DEB-TACE which indicates better response rates (Figure 2).

Results of this study displayed non-significant differences between BEADs and Lipiodol treated patients concerning; gender female to male ratio 4:1 respectively, age mean \pm SD 62.5 ± 0.2 , smoking habit, BCLC stage: Child-Pugh A total number 33 (82.5%) and Child-Pugh B total number 7 (17.5%), hepatitis C which was predominant 35 (87.5%) and hepatitis B 5

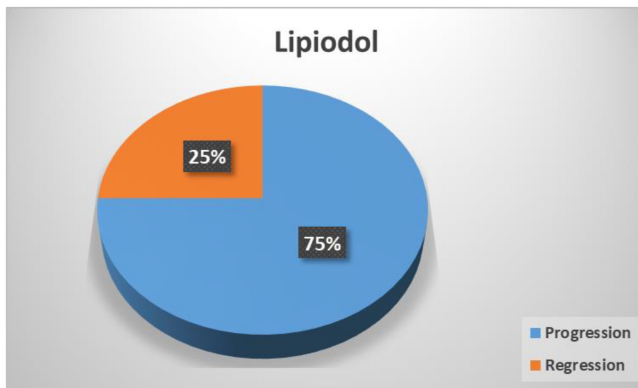
Table 7. Progression and regression of in HCC patients treated with trans-arterial chemo-embolization (TACE) using doxorubicin-Lipiodol vs doxorubicin-loaded drug-eluting beads

Characteristics	Total	Type of treatment (N, %)	
		Lipiodol	Beads
Progression	23 (57.5%)	15 (75%)	8 (40%)
Regression	17 (42.5%)	5 (25%)	12 (60%)

Source(s): Table by authors



(a)



(b)

Source(s): Figure by authors

Figure 2. Drugs response rate, a: Beads, b: Lipiodol

(12.5%) and another comorbidity. Meanwhile, a significant increase in family history 19 (95%) in Lipiodol treated patients compared to BEADs treated patient 9 (45%) with p -value = 0.003, our result agrees with [Weledji \(2018\)](#) which states that a family history of liver cancer increases HCC risk, independently of hepatitis. According to [Liu et al. \(2018\)](#), The baseline clinical characteristics were similar between treatment groups also according to [Wu et al. \(2018\)](#) and [Chen et al. \(2021\)](#), At the reference point, there were not any significant differences between the treated groups in terms of patient demographics, tumour burden or health type. Our study confers with ([DiStefano, 2020](#)), which states that women at older age (postmenopausal women) experience high HCC than men.

4. Discussion

The present study aimed to evaluate the efficacy and safety of beads loaded with DOX compared to DOX loaded Lipiodol (cTACE) in the treatment of Egyptian patients with HCC. In addition, pharmacogenetic of genes related to drug deposition could predict the clinical outcomes.

Regarding the laboratory data, liver function, kidney function, international normalized ratio (INR) and the haematological data, there were insignificant differences observed between the two groups. On the other hand, HCC patients Alpha-fetoprotein showed a marginally significant increase in the median and interquartile range for BEADs treated patients 24.5 (13–53.5) compared to Lipiodol treated patients 10.2 (4.5–28.2) with P -value = 0.067.

The high concentrations of alpha-fetoprotein (AFP) in patients with hepatic carcinoma are a useful index for diagnosis, efficacy assessment and prognosis of HCC ([Zong, Fan, & Zhang, 2020](#)). AFP is a valuable tumour marker for examining of HCC after treatments ([Tarao et al., 2020](#)). It also may be variably elevated in the event of acute liver injury ([Zong et al., 2020](#)).

The outcomes of this study displayed a significant increase in the plasma peak concentration of Dox in Lipiodol treated patients with median and interquartile range compared to beads treated patients with P -value = 0.03. Such findings confirm the advantage of using the eluting beads rather than the Lipiodol emulsifier in minimizing the DOX. levels. According to [Nouri et al. \(2019\)](#), the embolic material should be optimal to achieve a reliable concentration of chemotherapeutic agents within the tumour tissues and a less systemic concentration, conjoined with the vessels supplying obstruction of the tumour. Since lipiodol is the most common medium used to administer the intra-arterial chemotherapy, behaves as a micro-embolic, contrast agent and as an indicator of tumour necrosis ([Letzen et al., 2021](#)). According to [Rossi \(2018\)](#), clinical experiments confirm a substantially lower serum concentrations and favourable intra-tumour chemotherapy after DEB-TACE compared with cTACE. Unlike lipiodol, DEB-TACE is better in regulating the occlusion level and anti-tumour drug releasing, which may ensure a prolonged and sustained medication delivery, besides a drug (Dox) high diffusion into the beads adjacent liver tissue ([Wu et al., 2018](#); [Zhao et al., 2020, 2022](#); [Zhao et al., 2020, 2022](#)). After the third cycle, the most significant reported side effects in this study were the cardiovascular symptoms detected in 8 (20%) of the total number of patients. Heart pain was a more significant side effect observed in patients treated with lipiodol 7 (35%) compared with 1 (5%) in patients treated with beads (P -value = 0.02).

Additionally, feeling dizzy, Gastrointestinal Toxicity (GIT), hair loss toxicity as well as feeling heat or cold intolerance toxicity were not statistically different between the two groups. Depression toxicity was more noticed in patients treated with lipiodol treated group 7 (35%) compared with patients 4 (20%) treated with BEADs. Moreover, the swelling of the face/lips and swelling lymph nodes were statically significant with Lipiodol patients 6 (30%) more than patients treated with BEADs patients 1 (5%). Also feeling unwell and inflammation side effect shows statistical significance, Lipiodol, 20 patients (100%) and

patients treated with BEADs 20 patients (100%) with (p -value 0.01) and (p -value 0.01) respectively. The advantage of using BEADs over cTACE regarding toxicity in this study may be due to slow DOX release into systemic circulation. Generally, all chemotherapeutic drugs are deliberately released into the tumour at an uninterrupted high concentration, that leads to synergistically blocked at the commencement of the treatment and at certain concentration, where the tumour cells are subjected to greater chemotherapeutic effects in comparison to the healthy perfusion (Zhao *et al.*, 2022).

Depression as stated by Zhang *et al.* (2017), when patients are diagnosed with cancer, it can alter the immune function of the patients and result in larger tumour progression. There may be a weakening of the arterial wall predisposing to aneurysm formation and rupture may occur due to the inflammation response (Zou *et al.*, 2019). Lots of detailed studies are dedicated to distinguishing innovative cancer diagnostics or cure schemes that would enhance efficacy and resurgence rates. For this reason, we decided to assess the contribution of 4 genes included an SNP: ABCB1 (C3435T); ABCG2 (G34A); GSTM1 and GSTT1 gene, to the Dox pharmacokinetics and clinical response in HCC patients. The pharmacogenetic variability in drug absorption, metabolism and interactions between prescribed drugs and/or other related factor patients including Gastrointestinal (GI) abnormalities patients or drug exposure and response affecting diseases (Gadisa, Assefa, Wang, & Yimer, 2020).

Adenosine triphosphate (ATP)-binding cassette (ABC) transporters are responsible in active pumping of various substrates throughout the cellular membrane (Amawi, Sim, Tiwari, Ambudkar, & Shukla, 2019). These transporters plays a role in the pathogenesis of respective human diseases (Amawi *et al.*, 2019; Juan-Carlos, Perla-Lidia, Stephanie-Talia, Mónica-Griselda, & Luz-María, 2021). Several genetic variants of ABCB1 have been expressed to affect the transporter expression and function. The overexpression of specific transporters has been considered as a crucial factor in the development of chemotherapeutic resistances. The ABCB1 gene encodes for a transmembrane glycoprotein (P-gp) capable of pumping DOX out of the tumour cell (Jaramillo, Al Saig, Cloos, Jansen, & Peters, 2018). Accordingly, ABCB1 gene was recommended as a possible determining factor of intracellular concentration of DOX through ATP dependent efflux pathway (Gillespie *et al.*, 2015).

There were no statistically significant findings found among ABCG2, GSTM1 and GSTT1 genetic polymorphisms in both lipiodol and BEADs treated patients; on the other hand, the present study indicates that chromosomal alterations and the genetic polymorphism of ABCB1 (C3435T) may have a significant role in modulating the HCC risk. Our results demonstrated that patients who have a homozygous CC and treated with BEADs represent 70.59%; while patients who have heterozygous CT and treated with lipiodol represents 75% (p -value = 0.004). Thus, the ABCB1 gene C3435T polymorphism could be a consistent predictor of response to DOX in HCC patients.

The scholarly work of Hoffmeyer *et al.* (2000) confirmed that the TT genotype of C3435T was concurrently associated with expression reduction of P-gp, and consequently reduces the cellular elimination of the chemotherapeutic drugs. Based on such findings, we assume that patients diagnosed with the ABCB1 3435TT genotype would be more susceptible to drug response in chemotherapy with higher survival advantage compared to ABCB1 3435 CC/CT. In our study, the frequency of efflux transporter ABCB1 (C3435T) gene homozygous TT allele percentage for the total number of patients is (7.5%) of patients treated with BEADs, while for lipiodol treated patients' homozygous TT 0 (0 %) patients showing statistical significance difference (p -value = 0.004).

Other research studies have reported a higher dose-adjusted in the ABCB1 C3435T T-allele carriers as compared to the homozygous CC genotype (Azam, Khan, Khaliq, & Bhatti, 2021). The factors accountable for bioavailability variation in the drugs could illustrates the behaviour of homozygous 3435CC variant of ABCB1 gene which is significantly expresses a lower expression of intestinal P-gp in comparison to other patients with ABCB1 3435TT

genotype. Accordingly, affecting the bioavailability of the genes (Fernando, Forbes, Angus, & Herath, 2019) still, well-designed, multi-centre and large-scale prospective studies are required to further confirm the validity of our results. Dai *et al.* (2014) have recommended the given survival advantage of cTACE treatment in HCC compared to conservative treatment. Nevertheless, the relatively high tumour recurrence rate after cTACE chemoembolization may possibly indicate a hepatic dysfunction and increase the liver toxicity, which inhibits the usage of TACE in particular HCC patients (Fan *et al.*, 2021). Furthermore, being a risk factor for HCC development, liver cirrhosis predisposes patients to portal hypertension (Lopez-Gomez *et al.*, 2021). According to Zhao *et al.* (2022), The Kaplan-Meier cumulative survival curve of the DEB-TACE group (22 months) was significantly longer than that of the cTACE group (17 months). Such findings were confirmed closely all subgroups of the current study population, may be correlated to the ability of DEB-TACE to spread further than cTACE into the sinusoids to the distal portal vein branches, passing through the pre-biliary plexus a transient dual embolization arterial as well as portals (Facciorusso, 2018). Drug-eluting bead has the advantage of permanent embolization of tumour blood supply arteries, by the mean of loading chemotherapy drugs and releasing them slowly in local regions (Wu *et al.*, 2018).

5. Conclusions and recommendations

In conclusion, the current study provides valuable insight into the effectiveness of Doxorubicin for treating HCC in Egypt. The findings indicate that the drug is more effective in treating older female patients, which has important implications for treatment strategies and future research in this area. For healthcare providers in Egypt, the study underscores the importance of considering age and gender when developing treatment plans for patients with HCC. The most significant factors affecting Dox response in Egyptian HCC patients were the plasma level of Dox, in addition to SNP of efflux gene (ABCB1). The ABCB1, C3435T genotype (CT) and (CC) were significantly associated with Dox concentration. The progression manner under DEB-TACE treatment was mostly local and intrahepatic, with a longer RR observed under DEB-TACE treatment in the study. Consequently, the cTACE independent role as an indicator of postponed progression was proven in the Log-rank test, which demonstrated the cTACE was lower than DEB-TACE in which the latter expresses a better response rate. In line with other analysis, the group analysis showed a survival benefit after DEB-TACE over cTACE in patients demonstrating features commonly associated with additional aggressive tumours. Therefore, it is recommended to extend investigations in a future prospective study with a larger patients' population to validate these findings.

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