PROTEIN INDUCED VITAMIN K ABSENCE(PIVKA-II) RESPONSE IN ASSOCIATION TO TREATMENT MODALITIES AMONG HEPATOCELLULAR CARCINOMA PATIENTS AT HOSPITAL UNIVERSITI SAINS MALAYSIA.

DR ADIL ANVARALI DATOO

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR MASTER OF MEDICINE (RADIOLOGY)

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ACKNOWLEDGEMENT

Such a magnitude of study would not have been possible without the guidance, participation, kind gesture, assistance and cooperation from a lot of people, most of whom helped out of their free will with no incentives. Without their contributions, this study would not come to fruition and I will be eternally grateful to all the people who have helped me in some form, in one way or another. Without the willingness of the patients, this study would have never been completed and they deserve the utmost credit for the study and a heartfelt appreciation for helping me complete this study.

I would like to express my deepest and utmost appreciation particularly to the following:

1. Dr Nur Karyatee Binti Kassim, the corresponding author and Associate Professor Dr Mohd Shafie Abdullah who is my mentor for this study, both the aforementioned doctors have helped me tremendously throughout the course of this study and beyond with their helpful insights, easy approachability and all in all being wonderful supervisors for this study.
2. Dr Julia Bt Omar, Professor Dr Lee Yeong Yeh and Dr Norhanan Binti Hamzah for their valuable time for this study and their valuable insights.
3. Prof Madya Dr Mohd Ezane Aziz, Prof Madya Dr Juhara Haron, Dr Khairil Amir Sayuti, Dr Chandran Nadarajan, Dr Wan Aireene Wan Ahmed, Dr Hadif Zaidin Samsuddin, Dr Ahmad Tarmizi Mus, Dr Nur Asma Saipiai and Dr Nasibah Mohamad all of whom directly or indirectly contributed their ideas and comments to the success of this study.

4. My colleagues at both the department of radiology and also the department of chemical pathology who helped me in every possible way to help complete this study.

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LIST OF ABBREVIATIONS

AFP- Alpha Fetoprotein

BLCS- Barcelona liver cancer staging.

CCRT- Combined chemoradiotherapy

CR- Complete response

CT- Computed tomography

DCP(PIVKA-II)- Des-gamma-carboxy-prothrombin

HCC- Hepatocellular Carcinoma

HUSM- Hospital USM

mRECIST- Modified Response Evaluation Criteria in solid tumors

MRI- Magnetic resonance imaging

MWA- Microwave ablation

NASH- Non-alcoholic steatohepatitis

PD- Progressive disease

PEI- Percutaneous alcohol injection.

PIVKA- II – Protein induced Vitamin K absence/antagonist II

PR-Partial response

RFA- Radiofrequency ablation.

SD -Stable disease

sd- Standard deviation

SPSS- Statistical product and service solution.

TACE- Trans-arterial chemoembolization.

PROTEIN INDUCED VITAMIN K ABSENCE(PIVKA-II) RESPONSE IN RELATION TO TREATMENT MODALITIES AMONG HEPATOCELLULAR CARCINOMA PATIENTS AT HOSPITAL UNIVERSITI SAINS MALAYSIA.

ABSTRACT

Background: Hepatocellular carcinoma(HCC) is the commonest tumour of the liver with multiple aetiologies responsible for cirrhosis which evidently and eventually leads to hepatocellular carcinoma. With drastically increasing incidence since 1990 in Malaysia from 6.1 to 42.8% and with an annual mortality rate of 6.1%, the diagnosis of hepatocellular carcinoma is mainly dependent of imaging and biochemical markers. The commonest imaging modalities used to diagnose hepatocellular carcinoma are magnetic resonance imaging and computed tomography of the liver, while the commonest tumour marker used in conjunction with imaging is alpha-fetoprotein(AFP).In the setting of hepatocellular carcinoma, 30% of the time, the tumour marker alpha-fetoprotein is not elevated which leads to a conundrum during the time of diagnosis. A more robust tumour marker is required and PIVKA-II has shown potential in this regards with a few studies showing that PIVKA-II was superior in both sensitivity and specificity in the initial diagnosis, during follow up post treatment via hepatic artery embolization and hepatic artery infusion and it also shows a

better correlation with overall survival in comparison to AFP. The purpose of this study is to evaluate the association between radiological response and PIVKA-II response among patients who undergo interventional radiological treatment, as radiological response is used as a bench mark to either proceed with more interventions or to observe the patients.

Methods: A prospective study was conducted at Hospital Universiti Sains Malaysia(USM), Kota Bharu, Kelantan, Malaysia, where 66 patients who underwent interventional radiological treatment for hepatocellular carcinoma had blood investigation for PIVKA-II taken prior to the treatment, 6 weeks post treatment and 3 months post treatment with concurrent computed tomography or magnetic resonance imaging at baseline, 6 weeks post treatment and 3 months post treatment. Of the 66 patients, only 28 patients were available at the end of the 3rd month post intervention, this was due largely to the patients succumbing to their disease and partly due to the pandemic. The radiological response was based on modified response evaluation criteria(mRECIST) into four criteria's of progressive disease, stable disease, partial response and complete response. PIVKA-II response was classified into either PIVKA-II responders or non-responders. The association between radiological response and PIVKA response was carried out using Fischer exact test.

Results: There was a significant association between radiological response and PIVKA-II response at 6 weeks post interventional radiological treatment with a P value of <0.001 and a Cramer's V value of 0.71. However at 3 months post

treatment, there was no significant association between radiological response and PIVKA-II response with a P value of 0.915 and Cramer's V value of 0.141.

Conclusion: The overall study shows that there was no significant association between post interventional radiological response at 3 months and PIVKA-II response and as of now, tumour marker PIVKA-II in itself cannot be a substitute for radiological imaging. However, it can be used in conjunction with imaging.

Keywords: HCC, PIVKA, AFP, CT LIVER, MRI LIVER, mRECIST, TACE, PEI, RFA, HUSM

RESPONS PROTEIN INDUCED VITAMIN K ABSENCE(PIVKA-II) BERHUBUNGAN DENGAN MODALITI RAWATAN PADA PESAKIT BARAH HEPATOCELLULAR DI HOSPITAL UNIVERSITI SAINS MALAYSIA.

ABSTRAK

Latar belakang: Barah hati adalah ketumbuhan hati yang paling biasa dengan kejadiannya meningkat walaupum dengan pelbagai etiologi yang bertanggungjawab menyebabkan sirosis yang akhirnya membawa kepada kanser hepatoselular. Malaysia mempunyai kadar kematian tahunan 6.1% yang meningkat secara drastic sebanyak 42.8% sejak tahun 1990. Diagnosis barah hati bergantung kepada pengimejan dan penanda biokimia iaitu alfa-fetoprotein yang paling banyak digunakan. Walaubagaimanapun dalam sesetengah barah hati, alfa-fetoprotein tidak meningkat dalam 30% kes, emnyebabkan dilemma dalam diagnosis barah hati dan dengan mengambil kira sifatnya yang berubah-ubah dan kurang kebolehpercayaan penanda kanser yang lebih tepat diperlukan untuk mengiagnosis dan semasa rawatan susulan pesakit. PIVKA-II telah menunjukkan potensi dalam HCC melalui kajian yang menunjukkan kebolehpercayaan dalam diagnosis dan rawatan susulan pesakit HCC pasca rawatan.

Methodologi: Kajian prospektif dilakukan di Hospital Universiti Sains Malaysia yang melibatkan semua pesakit yang didiagnosis menghidap barah hati.

Pengukuran asas penanda kanser PIVKA-II dilakukan semasa diagnosis dengan pengukuran imbasan CT pada fasi arteri CT hati 4 fasa. 6 minggu selepas rawatan, sampel darah PIVKA-II dan pengukuran CT pada fasa arteri dilakukan dan rutin yang sama diulangi 3 bulan pasca rawatan.

Keputusan: Sebanyak 66 pesakit direkrut untuk kajian ini dan 66 sampel diperoleh pada awal dan pada 6 minggu selepas rawatan, namun pada selang 3 bulan hanya 28 sampel yang diperoleh kerana keadaan pandemic yang tidak dapat dijangkakan dan pesakit yang meninggal dunia akibat penyakit itu sendiri. Tindak balas radiologi dan tindak balas penanda kanser pada 6 minggu dan 3 bulan masing masing menjunjukkan nilai P <0.001 dan 0.915. Ini menunjukkan bahawa terdapat hubungan positif antara pemboleh ubah bebas pada 6 minggu tetapi tidak ada hubungan yang signifikan pada 3 bulan.

Kesimpulan: Tidak balas radiologi dan tidak balas penanda kanser pada 6 minggu dan 3 bulan masing masing menjunjukkan nilai P <0.001 dan 0.915. Ini menunjukkan bahawa terdapat hubungan positif antara pemboleh ubah bebas pada 6 minggu tetapi tidak ada hubungan yang signifikan pada 3 bulan

Kata Kunci: Kanser Hati, PIVKA-II, AFP, TACE, RFA, MWA, Hepatektomi, CT hati 4 fasa.

CHAPTER 1: BACKGROUND

1.1 Introduction

Liver is the second largest organ within the body and constitutes 1.5 to 2.5% of lean body weight of an adult human being with a variety of functions including excretory and secretory, vascular, immunological and metabolic functions. Due to its strategic location between the digestive tract and general circulation, it plays a major role in metabolism, distribution of nutrients and converting toxic metabolites and xenobiotics to non-harmful detoxified substances(Alamri Z, 2018). It is the source of most of the clotting and inhibitors, the liver protects against both bleeding and unnecessary activation of coagulation cascade.(3) As no other organ can compensate for all these functions performed by the liver, disease of the liver can be quite devastating(Chiang J, 2014).

The eighth most common cause of cancer for both genders throughout the world is Hepatocellular carcinoma, while in Malaysia hepatocellular carcinoma is the fifth most common cancer amongst males and eighth most common for females. The annual mortality rate per 100,000 people from liver cancer is 11.4% globally, 12.1% in Southeast Asia. While in Malaysia the annual mortality rate was 6.1% in the year 2013, which has risen dramatically by 42.8% since the year 1990. In terms of the case of years of life lost, liver cancer has risen from 26th in the year 1990 up to 22nd in 2010 with the annual years of life lost from liver cancer increasing by 31.5%(Mohamed R *et al,* 2018).

Studies carried out locally by the University Malay medical centre which included 115 patients who had presented to their hospital in 2013 showed that the mean age of diagnosis was 61.14(±1.11) with the vast majority of the population being of male gender(n=92). In terms of race, the majority of the patients were of Chinese ethnicity(n=69), which represented 60% of the sampled population followed by Malays(n=33) which represented 28.7% of the population , and then Indians(n=13) which was 11.3% of the population. The commonest aetiology within the study for hepatocellular carcinoma was hepatitis B virus which accounted for 51.3% while 9.6% of the patients had chronic hepatitis c virus(Mohamed R *et al*, 2018).

Studies carried out locally by the University Malay Medical Centre(UMMC) , which included 115 patients who had present to their hospital in 2013, showed that the mean age of diagnosis was 61.1(±1.11) with the vast majority of the population being of male gender, while in terms of race, the major of the patients were of Chinese ethnicity followed by Malays and then Indians with a percentage of 60.0%, 28.7% and 11.3% respectively. The commonest aetiology for cirrhosis and hepatocellular carcinoma was hepatitis B virus which accounted for 51.3% while hepatitis C accounted for only 9.6%(Mohamed R *et al*, 2018).

In Malaysia, for the longest time, the Hepatitis B virus has been the leading cause of hepatocellular carcinoma even though there has been a wide availability of an effective hepatitis B vaccine, while the burden of hepatitis C virus has been slowly rising within the country and this trend will eventually change in the coming decades. The number of hepatocellular carcinomas secondary to non-alcoholic steatohepatitis has also been on the rise in recent times with the prevalence of non-alcoholic fatty liver disease is estimated at 22.7% of the population and being highest amongst the Malay and Indian communities. As per the study carried out by University Malaya, cryptogenic and alcohol was the most common cause of hepatocellular carcinoma while for Malay and Chinese communities, HBV remained the major cause.[8]Hepatocellular carcinoma secondary to non-alcoholic steatohepatitis has also been on the rise in recent times with the prevalence of non-alcoholic fatty liver disease is estimated at 22.7% of the population and being highest amongst the Malay and Indian communities. As per the study carried out by University Malaya, cryptogenic and alcohol was the most common cause of hepatocellular carcinoma while for Malay and Chinese communities, HBV remained the major cause(Mohamed R *et al*, 2018).

Barcelona clinic liver cancer system(BCLC) is used to stage HCC and the study which was conducted at UMMC in 2013 showed that about 40% of the patients presented at the stage D where only palliative care can be offered, while only 17% of the patients came at a very early stage, stage A. Stage B had a total 22% of patients and stage C which in terms of treatment is similar to D where only palliative care can be offered had a total percentage of 20%. This study showed that patients normally presented more often in later stages where only palliative care can be offered. While an earlier presentation in stage A or B offers the patient a lot more treatment options

ranging from hepatectomy for A to TACE, RFA, PEI, MWA for multicentric lesions in A and B.[26] Treatment modalities can be divided into transplantation, surgical resection, systemic therapies, radiation therapy, local ablative therapies, and chemoembolization. The management of HCC is guided by the tumor staging, reserve liver function and patient performance status which are the parameters used in BCLC(Arslangolu A *et al*, 2016).

Diagnosis of HCC is normally based on tumour marker, namely AFP with supporting radiological features on either contrasted ultrasound, CT scan, or MRI with typical CT and MRI features of HCC. Tumour markers used in diagnosis of HCC have conventionally been AFP, however, this can be within normal limits in 30% of the patients. Typical features of HCC include hyperenhancement of the lesion on arterial phase with rapid washout seen on port venous phase. Other ancillary findings which may be helpful include tumour capsules seen in approximately 70% of the cases and HCC can show expansile growth. The rim enhancement observed in PV or arterial phase is considered to the capsule. Another finding suggestive of HCC is a nodule in nodule appearance, that is a presence of a smaller inner nodule that shows different imaging features from the one surrounding it. Portal vein invasion is common HCC and is seen in up to 44-62.8% of the cases. Both bland thrombus and tumoral thrombus will show portal vein enlargement and filling defect on portovenous phase, however, the bland thrombus will not enhance on portovenous phase while the tumoral thrombus will enhance on portovenous phase. Atypical hepatocellular carcinomas normally pose a diagnostic dilemma and can be classified into non-hypervascular HCC, HCC with targetoid appearance, fibrolamellar HCC, and infiltrative HCC and intraductal

growing HCC. These tumours are better evaluated by MRI rather than CT and will frequently require pathological correlation(Arslangolu A *et al*, 2016).

As hepatocellular carcinoma is a widespread disease, a multidisciplinary approach is normally adopted for these patients. There are multiple arrays of treatment including radiological, surgical, and pharmacological. Recently, image guided transcatheter and ablative approaches under interventional radiology currently gaining a lot of traction(Arslangolu A *et al*, 2016).

On the basis of prognosis, multiple studies have found that PIVKA-II and not serum AFP was a valuable independent prognostic factor in HCC(Kim J *et al*, 2013 and Kim H *et al* 2009). Comparing AFP and PIVKA-II in terms of follow up after hepatectomy, it was seen that compared to change in AFP levels, normalization of PIVKA-II levels was more significantly associated with good patient survival after hepatectomy and that normalization of PIVKA-II reflected the efficacy of the treatment and is suitable predictor of prognosis in HCC patients with a P value of 0.008 at 2500 days post hepatectomy and P value of <0.001 for overall survival percentage at 2500 days(Cerban R *et al*, 2018 and Nanashima A *et al*, 2006).

The tumour marker PIVKA-II is not widely used an available in our clinical setting however it has shown to be very in differentiating HCC patients from healthy individuals based on a prior study conducted at HUSM (Karyatee K *et al*, 2020), however it has not been studied in the current setting beyond one month post interventional treatment where it showed a good association with radiological response. It is clinically important to have a tumour marker would help in detecting recurrence even when the recurrence is radiologically occult so that an earlier treatment therapy can be arrange for or if there is a strong association between the

tumour marker and the radiological response, the tumour marker can serve as a substitute to imaging in order to avoid radiation.

1.2 Objectives

1.2.1 General Objectives

To study the relationship between radiological response and PIVKA-II response post-interventional radiological treatment for hepatocellular carcinoma.

1.2.2 Specific Objectives

1. To determine PIVKA-II response at 6 weeks and 3 months post interventional radiology treatment.

2. To determine radiological response on CT scan post interventional radiological treatment at 6 weeks and 3 months.

3. To determine association between radiological response and PIVKA-II response among patients who undergo interventional radiological treatment.

1.3 Hypothesis

There is a positive association between PIVKA-II levels and radiological response among patients who undergo interventional radiological treatment.

1.4 Research Question

What is the association between PIVKA-II response and radiological response post interventional radiological treatment?

CHAPTER 2: LITERATURE REVIEW

2.1 Epidemiology, aetiology, signs and symptoms of HCC.

Hepatocellular carcinoma is the commonest primary liver tumour worldwide and one of the leading causes of death globally in both genders. 75-90% of the patients with HCC have cirrhosis of the liver which is seen as an important risk factor for the disease. The risk of cirrhosis is between 1% to 5% in cirrhosis caused by hepatitis B and C. Other risk factors of cirrhosis include hemochromatosis, alpha-1-antitrypsin deficiency, cryptogenic and non- alcoholic steatohepatitis which eventually lead to cirrhosis. Other risk factors for cirrhosis which eventually leads to hepatocellular carcinoma include alcohol, alpha- one antitrypsin deficiency, hemochromatosis, cryptogenic and non-alcoholic steatohepatitis.[1]The number of hepatocellular carcinomas secondary to non-alcoholic steatohepatitis has also been on the rise in recent times with the prevalence of non-alcoholic fatty liver disease is estimated at 22.7% of the population and being the highest amongst the Malay and Indian communities. As per the study carried out by University Malaya, cryptogenic and alcohol was the most common cause of hepatocellular carcinoma while for Malay and Chinese communities, HBV remained the major cause(Mohamed R *et al*, 2018).

In the background of cirrhosis, hepatocellular carcinoma is thought to develop in a sequence Starting from the regenerative nodule to dysplastic nodules and then

going on to become hepatocellular carcinoma, however, hepatocellular carcinoma can occur in de novo. While regenerative nodule is not premalignant and a dysplastic nodule is premalignant, both derive their bloody supply from the portal vein. Hepatocellular carcinoma on the other hand derives its blood supply from the hepatic artery(Ogunwobi o *et al*, 2019).

Hepatocellular carcinoma patients typically present in one of two forms, commonly with underlying cirrhosis who develop deterioration of their liver function and present with worsening ascites, jaundice, or hematemesis secondary to variceal bleeding, anorexia or abdominal pain. While another subset of patients presents during screening with a high risk of hepatocellular carcinoma, as these patients are detected at a much earlier stage of the disease, the outcome of these patients is better in comparison to the earlier mentioned subset(Ogunwobi o *et al*, 2019).

2.2 Imaging Modalities used to assess Hepatocellular Carcinoma

Common imaging modalities used to assess and screen for hepatocellular carcinoma are ultrasound, computed tomography, and magnetic imaging resonance scans(Roberts L *et al*, 2018). Imaging algorithm of a suspected hepatocellular carcinoma patient or patients with high risk of HCC as suggested by the American Association for the Study of Liver Diseases(AASLD) dictates that nodules smaller than 1 centimetre should be evaluated with ultrasound at 3 months intervals with routine 6 month surveillance resuming once there is a documented stability of the lesions for 2 years . Nodules larger than 1 cm should be further evaluated with

multiphasic CT or MRI, with arterial hyperenhancement and venous or delayed phase washout being diagnostic of HCC. For indeterminate lesions on CT such as no arterial hypervascularisation or venous phase washout, a dynamic MRI is the next modality of choice. However, if such lesions are also indeterminate on MRI, biopsy is indicated(Roberts L *et al*, 2018).

On ultrasound, hepatocellular carcinoma has a multiple spectrum of findings, however, a visualized lesion or mass in a cirrhotic liver is considered hepatocellular carcinoma until proven otherwise. High doppler flow maybe present, especially at the periphery of the lesion or mass(Roberts L *et al*, 2018).

Basic protocol of computed tomography for hepatocellular includes multiphase CT scan including plain study, arterial phase study, portal venous phase study, and delayed imaging. The classical pattern of hepatocellular carcinoma is typically hypodense to the surrounding liver on the plain study, shows arterial phase enhancement of the lesion on arterial phase and the lesions becomes indistinct or hypoattenuating to the liver parenchyma on portal venous phase(Yaghmai V *et al*, 2013).

The imaging features on MRI for hepatocellular carcinoma show a slightly hyperintense signal on T2-weighted images relative to the surrounding liver and show a similar enhancement pattern to that of computed tomography on arterial phase with rapid washout of contrast in portal venous phase with gadolinium-based contrast agents. Rapid washout of contrast is very suggestive of malignancy as this feature is not seen on regenerative and dysplastic nodules(Yaghmai V *et al*, 2013).

2.3 Imaging features of Hepatocellular carcinoma on CT and MRI.

In reference to hepatocellular carcinoma, computed tomography has a sensitivity and specificity of 74% and 81% respectively while that of MRI is 81% and 85% respectively(Arslanoglu, A *et al* 2016).

Computed tomographic utilises multiphasic examinations in which images are acquired before (precontrast) and after contrast agent administration. Three enhanced phases typically are acquired: late hepatic arterial, portal venous, and delayed phase. Arterial phase is characterized by enhancement of the hepatic artery and its branches, and it is a critical phase in detection and characterization of hepatocellular carcinoma as all HCC derive their blood supply from the hepatic artery unlike the dysplastic nodules and regenerative nodules whose main blood supply is from the port venous system. Hence the arterial phase coincides with peak arterial perfusion and enhancement of hepatocellular carcinoma. Portal venous phase coincides with peak parenchymal enhancement, is characterized by enhancement of hepatic veins as well as portal veins, and is acquired at around 60–80 seconds after the start of contrast agent injection, during this phase, hepatocellular carcinoma shows washout of contrast, while regenerative nodules and dysplastic nodules show enhancement during this phase. Delayed phase is acquired at 3–5 minutes and its critical for characterizing key imaging features of HCC such as washout appearance and capsule appearance(Arslanoglu, A *et al*, 2016).

(Images adapted from Arslanoglu, A *et al*, 2016)

Figure 1

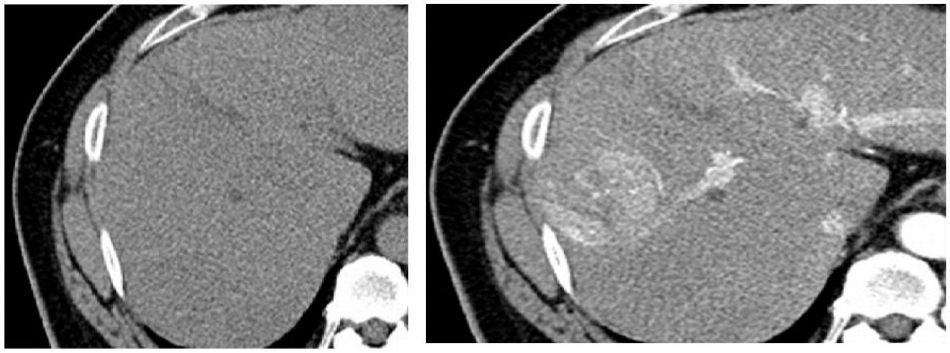

Fig A. Above, shows no discernible Lesion on plain study

Fig B. on Arterial phase there is Presence of enhancement.

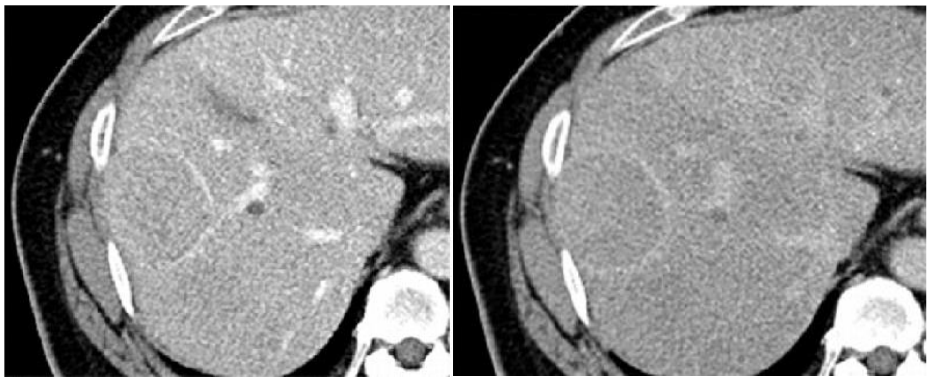

Fig C. shows washout on portovenous Phase.

Fig D shows an obvious capsule on delayed phase.