Comparing PET metabolic parameters with clinicopathological factors in predicting onset of early recurrence in recently diagnosed hepatocellular carcinoma

Original Article

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ABSTRACT

Introduction: Early recurrence of hepatocellular carcinoma (HCC) is a major risk factor affecting survival even after hepatectomy. Many clinical, biochemical parameters and pathological grading like fibrosis 1 index have been used for risk stratifying HCC. However not many studies have combined all of them. It is therefore important to risk stratify HCC especially with newer PET based metabolic parameters to see if they match with existing clinicopathological parameters to achieve better clinical outcome. The objectives of this study were twofold; firstly, to evaluate [^{18}F]FDG PET as a prognostic biomarker to predict tumour recurrence. Secondly, if clinicopathological parameters combined with PET indices increase the risk correlate in predicting HCC disease recurrence.

Methods: Records of 200 adult HCC patients were analysed, (6:1, Male: Female; mean age ± SD, 52 ± 2 year). All underwent [^{18}F]FDG PET (PET MR: PET CT = 168:32) and subsequent therapy. Patients had a follow up for at least 15 months or onset of first recurrence, whichever was earlier. Clinicopathological data, alpha-fetoprotein (AFP) titres, SUVmax and few other PET indices were documented along with details of first recurrence. Statistical analysis was also performed.

Results: In a multivariate analysis of various prognostic factors including T (SUVmax)/ L (SUVmax), serum alpha-fetoprotein, T stage, size of tumour, and vascular invasion of tumour, T (SUVmax)/ L (SUVmax) was the most significant with a cut off value of 1.9. Only vascular invasion of tumour and AFP titres had additional significance. 16% (32/200 patients) developed recurrence (OR 1.673). Comparing the low and high AFP titres by Kaplan Meir curve, P was found to be 0.039 that predicted a worse prognosis in patients with higher AFP titres. Similarly patients with higher SUV T/L: ratio of tumour SUVmax to liver (> 1.9) also revealed higher recurrence rate. Cut-off SUVmax was 3.03 g/ml in our series (range 2.5 - 23.8 g/ml) and found to be strongly associated with AFP, tumour size, number, and histological grade of tumour.

Conclusion: Our study shows that PET based metabolic indices are effective robust tools to predict tumour recurrence in aggressive HCC. Secondly, when clinicopathological parameters are combined with PET based indices there is better prediction of HCC recurrence and one can reclassify HCC patients into mild, moderate, and high-risk groups. We found it very useful in predicting poor clinical outcome especially in high-risk HCC patients; so that stricter surveillance measures can be recommended to identify early recurrence and offer appropriate therapy. The strength of this study lies in the fact that the observed associations between the combined parameters were found to be stronger than those reported in the past.

Key words: Hepatocellular carcinoma; Alpha-fetoprotein; Cirrhosis; Histology; SUVmax

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a major health issue and a major cause of cancer mortality [1] and ranked fifth most common cancer worldwide [2]. Incidence of HCC is rising in the developing world with a shifting incidence based on geographical regions that may be attributed to the changing prevalence of hepatitis, availability of advanced imaging techniques and molecular tools. As per literature evidence published in 2013, there is an annual increase in 22,000 new cases [2]. Effective screening strategies for HCC especially those with high-risk factors are valuable to guide more specific personalized management. 70-80% of all HCCs are related to Hepatitis B virus infection, other causes include alcohol, diet and cirrhosis etc [2, 3]. As per GLOBOCON 2018 reported by International Agency for Research on Cancer, number of new cases of liver cancer was 4.7% (841,080 population) annually worldwide [4]. Co morbidities like chronic liver disease, cirrhosis, and viral hepatitis can also add to the high mortality encountered in Indian patients with HCC. Early recurrence of HCC is a major risk factor affecting survival even after hepatectomy. Prognostic modelling in HCC is complex because underlying diseases and residual liver function needs to be accounted. Hence, the search is on to identify new robust biomarkers to better prognosticate HCC. It can be noted that many clinical indexes of liver dysfunction like child pugh score (CP), model for end stage liver disease score (MELD) and score based on serum albumin and bilirubin (ALBI) have been studied as a predictor for survival. Similarly, biochemical parameters like alpha-fetoprotein (AFP), and pathological grading like fibrosis 1 index have also been used for risk stratifying HCC. However, very few studies have compared clinicopathological and metabolic variables in HCC.

Serum AFP is the most widely used serological marker for screening and diagnosis of HCC, but nonspecific and not always reliable [5]. Normal AFP titres fall in the range of 5–10 ng/mL. Its values vary in different population of HCCs, and are elevated in testicular and ovarian malignancies as well as in benign conditions like chronic liver disease. European Association for the Study of the Liver (EASL) and the European Organization for Research and Treatment of Cancer (EORTC) consider AFP testing to be suboptimal for routine screening of early HCC (2B) [6]. It has also been found that about 80% of small HCC (< 2 cm) do not show high levels of serum AFP [7, 8] and many remain as non-secretors, i.e., do not secrete high levels of AFP in spite of proven HCCs [9]. Therefore, search is ongoing to identify the right set of variables that can be easily obtained to prognosticate HCC.

Not only diagnosis and surveillance of HCC but recurrence evaluation is also problematic and remains the major cause of death. This may be related to large tumour size, cellular dedifferentiation and early angioinvasion to name a few [10].

The objectives of this study were twofold; firstly, to evaluate ^{18}F-fluorine labelled Flurodeoxyglucose Positron emission tomography imaging ([^{18}F]FDG PET) as a prognostic biomarker to predict tumour recurrence. Secondly, if clinicopathological parameters combined with PET based indices increase the risk correlate in predicting HCC disease recurrence.

METHODS

This retrospective cohort study analysed data of 200 newly diagnosed HCC patients out of 970 patients who attended the hospital between Jan 2016 to Dec 2018. Only those with complete medical records were included.

Inclusion criteria

1. ≥ 18 years of age with recent histological confirmation of HCC
2. Tumour marker - baseline and follow-up serum AFP values
3. Serological marker of hepatitis - Hepatitis B surface antigen (HBsAg)
4. Investigations necessary for Scoring MELD (Model for End stage liver disease) and CP (Child Pugh) scores (for cirrhotics)
5. Patients showing abnormal increased [^{18}F]FDG uptake in primary liver lesion
6. Only those patients, who underwent therapy in any form, were included.

Exclusion criteria

Coexisting malignancy, previous therapy for other malignancies, benign liver diseases, pregnant or breast-feeding patients (relative contraindication for PET imaging) and patients with negative PET scan (i.e., no [^{18}F]FDG uptake in primary liver tumour).

For the conduct of study, data sourced from hospital records was used. All patients had a follow up for 15 months or until the documentation of first recurrence, whichever occurred first.

Clinicopathological data

Records of 200 newly diagnosed HCC patients showed mean age of 52 ± 2 years with male preponderance (6:1, Male: Female). Presenting complaints, smoking and alcohol consumption history, and details regarding onset of first tumour recurrence were documented. Baseline AFP and liver function tests were collected along with CP (a) good

hepatic function, (b) moderately impaired and (c) advanced hepatic dysfunction) / MELD scores (normal range 6-40) especially in cirrhotics. These clinical scoring systems were used to prognosticate the disease severity in our patients along with PET parameters. It is said that higher the MELD score, higher is the mortality related to liver disease. PET based glycolytic numerical index i.e. Standardized uptake value, SUVmax in units of gm/ml and metabolic parameters like ratio of tumour SUVmax to normal liver SUVmax = T SUVmax /L SUVmax, ratio of tumour SUV(max) to normal-liver mean, SUV (T SUVmax /L SUV mean were also calculated. Based on serum AFP values, patients were classified as low (0-399 μg/mL) and high (≥ 400 μg/mL) risk.

Imaging

All patients underwent [18F]FDG PET imaging (PET MR: PET CT = 168: 32 respectively). Whole body and regional liver [18F]FDG PET/MR were performed with gadolinium contrast [Biograph mMR (3T) - Biograph, Siemens Health Care Sector, Erlangen, Germany]. PET/CT was acquired using Siemens 16 slice Horizon PET/CT, Siemens Medical Solutions, Erlangen, Germany, with additional triple phase contrast abdomen CT (Computed tomography) as part of the PET/CT study. All patients underwent whole body (head to mid-thigh) imaging as per the standard recommendation along with high-resolution inspiratory chest CT (for lung nodules). 1 mCi (millicurie) per kilogram body weight of [18F]FDG was injected intravenously in euglycemic status after an overnight fast. Imaging was performed 1-hour post injection. Images were interpreted by senior nuclear medicine physician and radiologist.

Lesions with SUVmax of ≥ 2.5 were considered pathological. To evaluate [18F]FDG uptake, the region of interest (ROI) was drawn for each liver lesion, and the normal liver. SUVmax values were determined for all lesions. The ROI was drawn to encircle the highest [18F]FDG uptake of each tumour. For normal liver regions, two circular 1.5 cm-diameter ROIs were drawn in both lobes. SUV mean of normal liver was defined as the mean value of SUV mean of the ROIs. All tumour and non-tumour regions were defined by correlating PET with CT/ MR images. Macro and microscopic details of the primary lesion along with lymph nodal and distant metastases were tabulated.

Statistical analysis

Data of all included patients were tabulated using Microsoft-excel (version 16.0.6742.2048) and analysed using IBM SPSS (version 21, IBM Co., Armonk, New York, USA). Spearman's correlation coefficient (p) was used to analyse associations between recurrent and non-recurrent groups of patients for each of the variables. $P < .05$ was taken to indicate statistical significance. Logistic regression, Kaplan Meier methodology for survival function was assessed. Relative risk (RR), Hazard ratio (HR) OR Odds ratio (OR), 95% Confidence interval (CI) was calculated for variables analysed.

Ethical concerns

We used our hospital information system to source the medical data for every patient. Institutional Ethics Committee have approved this study conducted on human subjects. The study has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All patients have given their informed consent prior to their inclusion in the study. No personal or demographic details of patients were collected, and confidentiality was maintained.

RESULTS

Analysis of baseline patient data

In our sample population, mean age was 52 ± 2 years with male preponderance (6:1, Male: Female) (Table 1). Weakness, loss of appetite, abdominal pain, and weight loss were common presentations (33% patients). 19% were smokers while alcohol consumption was noted in 28% of patients (>80 g/day for 5 years or more). The serum AFP elevation in cirrhotic HCC patients in our series (median 440 ng/ml; range 4 – 90,453) was found to be significantly higher as compared to those without associated cirrhosis (median 134 ng/ml; range 2– 514) (P = 0.030) (Table 2). CP score, MELD scores (only in patients with ≥ 10 were considered) were analysed in relation to different AFP levels. MELD score ≤ 9 has lower mortality (1.9%), thus scores above 10 were included in our analyses. Based on these scores, patients were graded as low, intermediate, and high mortality. There was good association between each of these prognostic scoring systems with other parameters like AFP, SUVmax, different tumour sizes and tumour stages. Alcohol consumption showed association with HCC and HBsAg positivity (RR =1.7; 95% CI = 1.4 to 2.2) in our subjects. Qualitative variables were compared using chi square test. Quantitative variables were also analysed using correlation analysis.

Comparing with histology

Majority of primary HCC (67%) were multifocal in nature; right lobe was predominantly affected in our subjects. Histological grading of HCC was performed as per Edmondson – Steiner system [11] and cellular differentiation i.e., Grade 1, II, III, IV = Well differentiated WD, moderately differentiated MD, poorly and undifferentiated UD respectively.

Table 1: Baseline patient characteristics and tumour variables in recurrent and non-recurrent hepatocellular carcinoma.

Characteristics		Total n = 200	Recurrence n = 32	Non-recurrence n = 168	P value
Mean age±SD		52±2 yrs	59±6	55±6	0.14
Tumor numbers (median)		2.1±1.2	1.4±0.6	2.0±1.1	0.18
Tumor size		3.4±1.6	4.5±1.3	2.5±1.2	0.007
Serum AFP (µg/mL)		522±1450	1870±2700	100±43	0.001
T SUVMax / L SUV Max		1.4±1.2	1.90±1.57	1.08±0.06	< 0.001
Vascular invasion	Positive	19	11	08	0.023
	Negative	48	8	34	
Histology grades	I	22	5	76	0.37
	II	58	14	66	
	III	120	13	26	
T Stage	T1	124	16	114	0.76
	T2	52	9	42	
	T3	24	7	12	

Table 2: AFP levels with clinicopathological risk correlates.

Clinical features		AFP Low (0-399 ug/ml)		AFP high (> 400 ug/ml)		Total		Chi square value	P value
		N	%	N	%	N	%		
Tumor focality	Unifocal	45	22.5	21	10.5	66	33	0.932	0.334
	Multifocal	82	41	52	26	134	67		
Cirrhosis		54	27	32	16	86	43	5.430	0.362
Child pugh score in cirrhotics	A	44	40.7	20	18.5	64	54.75	12.467*	0.052*
	B	18	16.6	9	8.3	27	20		
	C	9	7.7	13	12	21	25.25		
MELD score ≥ 10		39	19.5	37	18.5	76	38	7.851	0.005
CLD with PHTN		57	28.5	32	16	89	44.5	0.551	0.459

Tumour staging was done according to AJCC (American Joint Committee on Cancer) guidelines. Higher baseline AFP titres were found in patients with multifocal HCC, which is comparable to literature [12]. Median number of total liver lesions were 2.1 ± 1.2 (recurrent group: non-recurrent group was 1.4 ± 0.6 and 2.0 ± 1.1 respectively). Tumour size was found to be larger in recurrent group (4.5 ±1.3 cm) than in non-recurrent group of patients (2.5 ± 1.2 cm).

Positive tumour margins were noted in 35% patients with Grade III/IV tumours. AFP in recurrent group was 1870 ± 2700 µg/mL when compared to non-recurrent group (100 ± 43 µg/mL) (P = 0.001). Vascular invasion was found in 11 patients in the recurrent group. Distribution of liver recurrent lesions were T1:T2: T3 = 16: 09:07, majority had smaller sized lesions. Micro invasion was analysed based on tumour focality, primary tumour size and histological grading. Histological grade was seen to be highly correlated with micro vascular invasion (P < 0.001)

(a) 11% patients were categorised under Grade 1 (WD), (b) 29% of patients as Grade II (MD) while, (c) 50% were found to be poorly differentiated or undifferentiated tumours (UD).

Portal vein thrombosis was seen in 19 patients. 57 patients with stage III/IV tumours had a higher association with AFP positivity (P < 0.001). The independent predictors of micro vascular invasion were tumour size greater than 4 cm (OR, 3.0, 95% CI, 1.2 to 7.1), and high tumour grade (OR, 6.3; 95% CI, 2.0 to 19.9).

Association with SUVmax

Three parameters for [18F]FDG uptake were studied. 1) Maximal standardized uptake value (SUVmax), 2) ratio of tumour SUVmax to normal liver SUVmax = T SUVmax /L SUVmax, 3) ratio of tumor SUV(max) to normal-liver mean, SUV (T SUVmax /L SUVmean were determined and used as prognostic factors and compared with clinicopathological parameters. SUVmax of primary liver lesion/s, nodal and distant metastases. [18F]FDG avid nodal deposits were noted in 20 patients; lungs were involved in 45 patients and other distant metastases was found in 61 patients in both groups of low and high AFP titres. Comparing the low and high AFP titres by Kaplan Meir curve, P was found to be 0.039 that predicted a worse prognosis in patients with higher AFP titres. Similarly patients with higher SUVmax, SUV T/L: ratio of tumour SUVmax to liver (> 1.9) also revealed higher recurrence rate.

The cut-off SUVmax was found to be 3.03 g/ml in our series (range 2.5 - 23.8 g/ml). This value was found to be strongly associated with AFP, tumour size, number and histological grade. Overall SUVmax and SUV mean of normal liver were 2.4 ± 0.5 and 2.1 ± 0.4, respectively. On receiver-operating-characteristic curve analysis, T SUVmax/L SUVmax showed the highest area under the curve, 0.887. The areas under the curve of T SUVmax/ L SUV mean and SUVmax were 0.885 and 0.730, respectively. The optimal cutoff values for T SUVmax / L SUVmax, T SUVmax /L SUV mean, and SUVmax were 1.9, 1.35, and 3.0, respectively (Figure 1).

From these results, T SUVmax / L SUVmax of 1.9 was used as the most effective prognostic factor on 18F [18F]FDG PET in the prediction of tumour recurrence. In the comparison between recurrence and non-recurrence groups, tumour size, serum AFP, vascular invasion, and T SUVmax/L SUVmax showed significant differences. In addition, these factors were determined as significant prognostic factors for tumour recurrence in the univariate analysis. However, in the multivariate analysis, only T SUVmax/ L SUVmax and vascular invasion were determined to be significant.

[18F]FDG PET has been established to be an important predictor of HCC treatment [11]. In our sample, tumour grade correlated and found to be statistically significant with T SUVmax/ L SUVmax (P = 0.37, P = < 0.001) similar to few studies in literature [13]. Furthermore, low and high AFP titres correlated with SUVmax (P = 0.450, P = 0.047 respectively).

Follow up

At follow-up, serum AFP, liver function tests were performed bimonthly. When recurrence was suspected, chest X-ray and whole body [18F]FDG PET/CT/MR were additionally performed to document recurrent liver lesion/s, nodal and metastatic deposits.

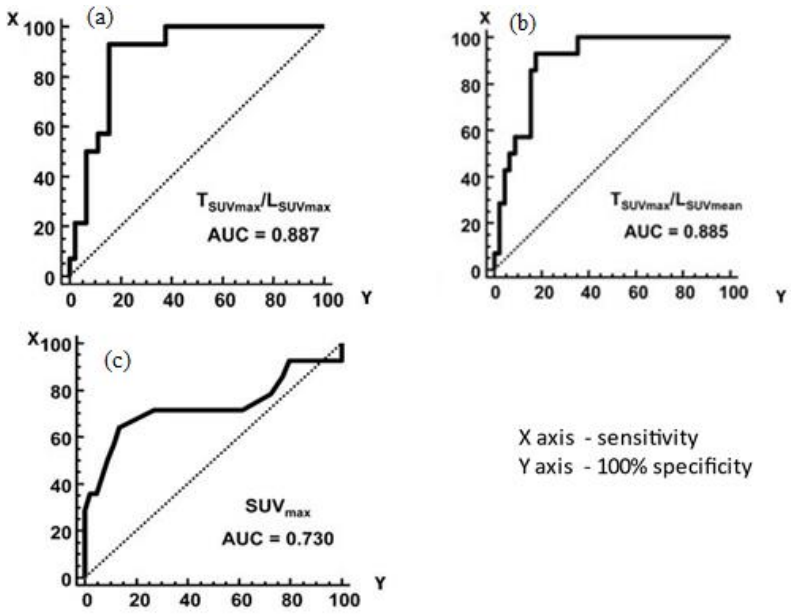

Fig 1. Predictive values of prognostic factors on [18F]FDG PET. T SUVmax / L SUVmax shows highest area under curve on receiver-operating-characteristic curve analysis (a). T SUVmax/ L SUVmean shows similar area under curve (b), but SUVmax shows significantly lower area under curve (c).

All our patients were subjected to some form of therapy (surgical: nonsurgical therapy = 72: 28% patients). 16% (32/200 patients) developed recurrence (OR 1.673). Scatter plot generated to highlight the AFP titres at baseline and first recurrence (Figure 2).

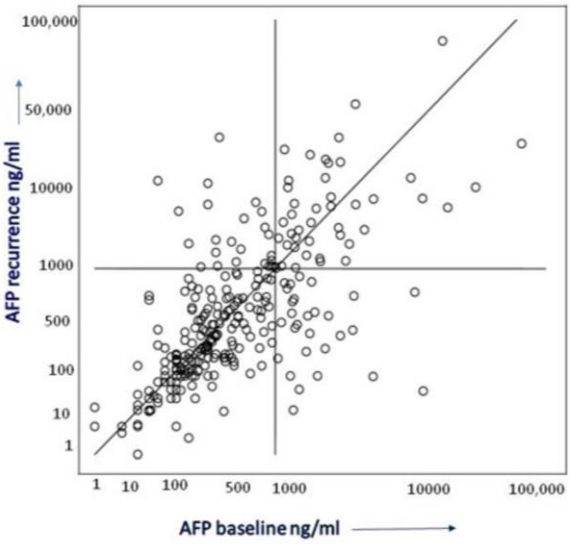

Fig 2. Scatter plot for alpha-fetoprotein titres in our hepatocellular carcinoma patients at baseline and first recurrence.

116 patients (58%) were alive at last follow up. 3 patients had transplant related complication. 1-year survival was found to be 86 %. Progressive HCC was the cause of death in 7 patients, and 9 patients died without evidence of recurrence (4 patients had cardiac problems, 1 had diabetic ketoacidosis, 1 died of renal failure and 3 died of metastases from other primaries). Kaplan Meir plot was generated (comparing the low and high AFP titres, P was found to be 0.039) that predicted a worse prognosis in patients with higher AFP titres. Similarly patients with higher SUVmax, SUV T/L: ratio of tumour SUVmax to liver (> 1.9) also revealed higher recurrence rate. According to T SUVmax/L SUVmax, the 15 months recurrence free survival rate above the cut off was markedly different from the rate below the cut off (97% vs. 57%, $P < 0.001$).

DISCUSSION

Hepatocellular carcinoma (HCC) is one of the major causes of mortality among patients with chronic liver disease worldwide. Our study shows a male preponderance. Li et al. found that the effects of sex hormones in the liver are *Foxa1*/*Foxa2*-dependent and provide a novel molecular mechanism responsible for gender dimorphism in HCC. Alcohol consumption has also been associated with baseline high serum AFP titres in HCC as evident in our series. It is an important modifiable risk factor as it induces hepatocyte injury and maybe a factor leading to cirrhosis and later HCC [14].

Elevated AFP and serum alkaline phosphatase levels indicate corroborative evidence for hepatocyte injury [15, 16]. In this study, there was a statistically significant correlation between tumour size and baseline serum AFP titres; and, between first recurrences, baseline serum AFP titres. Patients with AFP > 400 ng/ml have poor outcomes [17] and this is highlighted in our series. As seen in our study, other literature evidence has also established similar correlation between tumour size, recurrence, and baseline serum AFP titres [17, 18]. Larger the tumour dimensions, more the AFP secretion which was confirmed by Pawlik et al. [19]. In addition, it was stated that AFP induces tumorigenesis and metastases in mouse xenograft models [20].

Literature evidence states that high AFP values may indicate HBsAg positivity and related to direct carcinogenic effect of hepatitis B virus [21]. Like our study, high HBsAg titres have been associated with higher mortality in HCC patients [22]. While analysing prognostic scoring systems like MELD [12] and the CP scores in cirrhotics; higher MELD score calls for urgency in undergoing liver transplantation [22]. In our study, higher AFP values were associated with higher MELD scores.

We found that there is a statistically significant association between baseline serum AFP, tumour grade and microvascular invasion. Higher AFP values indicate greater degree of de-differentiation and angioinvasion [23]. Patients with baseline AFP level ≥ 400 ng/ml have approximately 4 times higher risk of pulmonary metastases [24]. However, we found extrahepatic skeletal deposits to be more common as cited by another study [25].

Glucose metabolism assessed on [18F]FDG PET is related to aggressiveness of HCC; higher the SUVmax higher the tumour grade [26]. [18F]FDG uptake in tumour cells is related to the expression of GLUT-1 and hexokinase II [27] (Figures 3 and 4).

Finally, observed associations between the combined parameters as stated above in our study, were found to be stronger than those reported previously [26, 28]. Our study showed that combined parameters can reflect the adverse outcome in HCC and can be used as surrogate markers for tumour characterization. Studies based on correlating imaging findings and histological associations of HCC have been varied and inconclusive [26, 28, 29]. A high AFP titre at the time of diagnosis is an indicator for further evaluation of other factors associated with HCC.

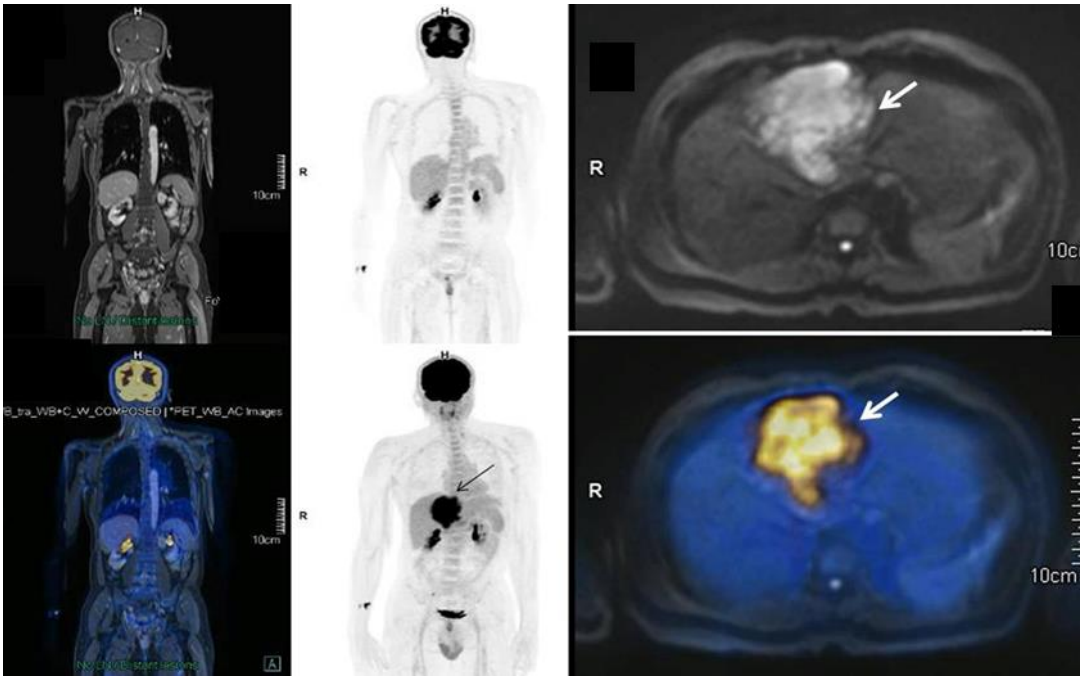

Fig 3. Whole body FDG PET/MRI (3T) in a 44-year-old male with biopsy proven poorly differentiated HCC. (Left) upper row: coronal MR, lower row: fused PET/MR. (Middle) MIP image, (Right) upper row: transaxial MR liver, lower row: fused PET/MR images. PET/MR imaging was performed using multiplanar T1, T2, STIR, DWI, Dixon and post contrast VIBE sequences and fused with PET images. Images reveal [18F]FDG avid large arterially enhancing lesion (arrow) in segment IVa / IVb of liver measuring 8.8 x 7.5 cm, extending into segment VIII and tiny lesions in segment II (SUVmax 3.4). There is associated [18F]FDG avid tumour thrombus occluding left portal vein and along the middle hepatic vein and infra diaphragmatic (IVC) Inferior venacava (SUVmax 18.8). No nodal or distant metastases noted.

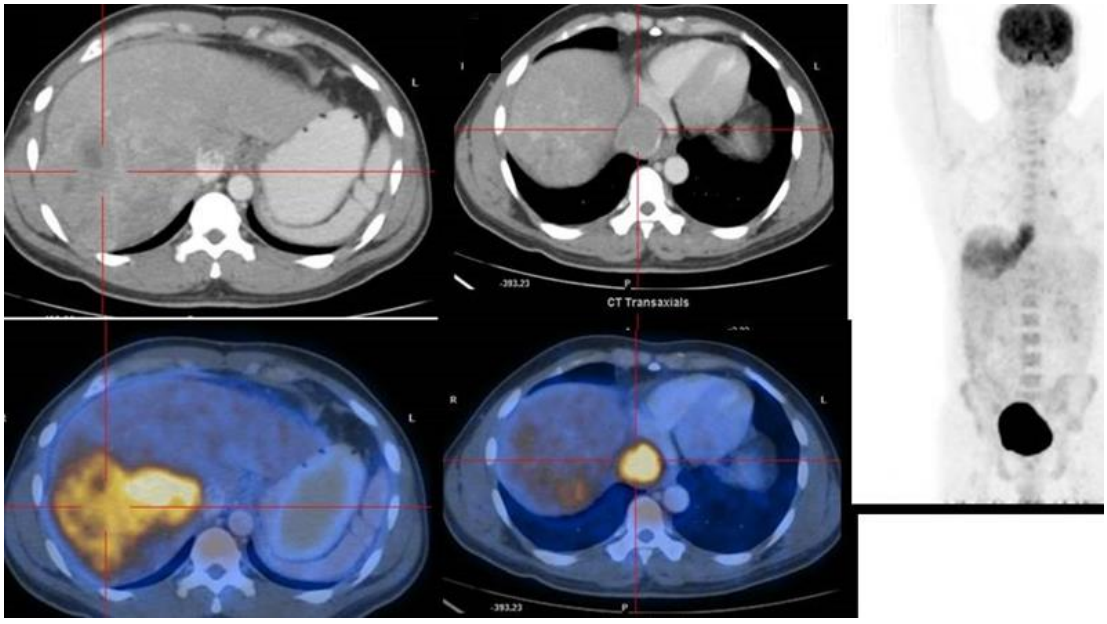

Fig 4. PET/CT images in a 45 year old male with HCC showing [18F]FDG avid right lobe primary HCC with IVC thrombus (Hepatitis Ag positive case). (Left) upper row: CECT liver transaxial, lower row: fused PET/CT transaxial image. (Middle) upper row: CECT and lower row: fused PET/CT transaxial image. (Right) MIP (Maximum intensity projection image) showing [18F]FDG distribution. Abnormal increased [18F]FDG uptake noted in large heterogeneously enhancing lesion (cross hair) involving right lobe of liver (9 x 10 cm) with necrotic component within it (SUVmax 7.0) and in filling defect in IVC ([18F]FDG avid tumour thrombus) extending up to right atrium (SUVmax 10.1) in middle image). No other [18F]FDG avid nodal or distant metastases.

Limitations

It is well known that well differentiated HCC may be [18F]FDG negative. Patients with [18F]FDG avid HCC were analysed in this study, so referral bias exists. Results of the study should be viewed in context of small sample size involved. The other limitation is the retrospective nature of data collection.

CONCLUSION

[18F]FDG PET is a single and important predictor of aggressive HCC and predicting recurrence. Higher the SUVmax relates to higher tumour grades. SUVmax and AFP titre were found to be independent risk factors predicting aggressive disease. Combining clinico pathological and metabolic data helps in further risk stratification of HCC. We found higher AFP values were associated with higher MELD scores. Other risk correlates were tumour grade and microvascular invasion. Of all the parameters studied, SUV T/L: ratio of tumour SUVmax to liver was the most significant in the prediction of tumour recurrence, with a cut off value of 1.9. It may be a good indicator for predicting poor clinical outcome in high risk HCC patients; therefore stricter surveillance measures can be recommended to identify early recurrence in this subgroup. The strength of this study lies in the fact that the observed associations between the combined parameters were found to be stronger than those reported in the past.

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