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ORIGINAL ARTICLE

Role of PET/CT in Postoperative Evaluation of Colorectal Cancer

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ABSTRACT

Background: Colorectal cancer (CRC) is the third most common cancer in men and the second in women worldwide, the current treatment for localized CRC is curative complete surgical resection after exclusion of distant metastases. Unfortunately, high recurrence rate was recorded post-operatively within 5 years. Early detection of recurrent CRC has become more important, as the treatment options for localized recurrence disease have improved significantly. **Objective:** to evaluate the role of PET/CT in postoperative evaluation of suspected recurrence or metastases in CRC patients in comparison with CECT. **Methods:** Between December 2018 and December 2019, twenty patients with history of surgical excision of CRC were evaluated for suspected local recurrence and metastasis. Both sexes without age predilection were included, medical, clinical history, any other follow up images, tumor markers (CEA), and pathological reports were reviewed for gold standard. **Results:** Local recurrence detected in 10 patients by PET/CT with 90% sensitivity, 90% specificity, 90% accuracy, 90% PPV and 90% NPV and detected in 9 patients by CECT with sensitivity 72.7%, specificity 88.8%, accuracy 80.0%, PPV88.8% and NPV 72.7%. While distant metastasis to different organs detected in 15 patients by PET/CT with sensitivity 87.5%, specificity 75%, accuracy 85%, PPV93.3%, NPV 60 % and detected in 12 patients by CECT with sensitivity 68.7%, specificity 75%, accuracy 70 %, PPV 91.6% and NPV 37.5%. PET/CT imaging detected 1 recurrent and 12 metastatic lesions that were missed by CECT. **Conclusion:** PET/CT is an efficient diagnostic imaging technique in detecting loco-regional recurrence and metastasis in patients with operated colorectal cancer with higher sensitivity and specificity, resulting in restaging and changing the management plane of the patients and avoiding unnecessary surgeries

Keywords: Colorectal cancer (CRC); Recurrence; Metastasis; Positron emission tomography (PET); Computed tomography (CT).

INTRODUCTION

CRC is the commonest GIT tumor; it appears to be one of the most widely diagnosed tumors despite sustained progress in diagnosis and treatment options it contributes significantly to many cancer-related deaths [1].

Abdominal pain, tenderness, loss of appetite, generalized weakness, weight loss, change in bowel habits, iron deficiency anemia and bleeding per rectum are most

common presenting symptoms of CRC [2-4]. It's more common between men, alcoholic, smokers and who suffers from chronic bowel diseases as chronic inflammatory bowel disease and FAP (familial adenomatous polyposis) [2].

Roughly about 55% of cases seen at rectosigmoid, 20% at the cecum, 15% at the ascending colon, 10% at the transverse and descending colon. Even so, there can be

possible differences in the site of origin based on age and gender [3].

Staging is commonly done by using TNM classifications system depending on depth of tumor invasion, lymph nodes involvement, metastasis to other organs as Primary Tumor size (T), regional lymph Node (N) and distant Metastasis (M) TNM staging is superior than Dukes staging because of greater information that may leads to stage migrations [4].

Metastatic disease was recorded in many patients; about 20% of individuals who are diagnosed with CRC have metastatic lesions at time of disease presentation. Metastasis occurs through different ways: lymphatic, hematogenous, contiguous or transperitoneal spread. Regional lymph nodes, liver, lung and peritoneum are most common sites of metastases from CRC [3-5] Fig (1-4).

Radical resection is the first management method, but recurrence and (or) distant metastasis reported in about 41 % of patients with in 3-5 years after surgery, so detecting any residual tumor cells, early recurrent tumors or distant metastatic lesion has become very important as the treatment options has improved significantly [6].

Traditional imaging techniques including contrast enhanced computed tomography (CECT) and magnetic resonance imaging (MRI), used for follow up. Diffusion MRI is a non-invasive functional technique can characterize tissues based on their water diffusion properties with different P-values and ADC measurement, it can differentiate recurrent masses from post-operative scar tissue and determine precisely the site and type of local recurrence. However, CT is mandatory in these patients as a screening modality and follow us. Can be used as early marker of treatment response as cell death and vascular alternative occurs before size changes [7].

Due to anatomical changes and tissue hyperplasia as post-operative sequelae conventional techniques may not be sensitive enough to identify low-volume lesions and differentiating between recurrence and post-surgical fibrosis as they depend on anatomical changes [8]. High carcino-embryonic antigen (CEA) serum level, used as a biological

marker of CRC recurrence, it was proved that it has an insufficient sensitivity and specificity and is therefore not helpful in the assessment of resect ability [9].

With the development of novel tracer and contrast agents imaging modalities has broadened specially with fusion of technologies as PET/CT positron emission tomography (PET) and Computed tomography (CT) [10-12]. PET/CT is a functional imaging modality using 2-[18F] fluoro-2-deoxy-D-glucose (FDG), which shows increased utilization and high uptake by malignant cells, it can successfully identify the metabolic activity of the equivocal or even missed by the other imaging modalities [11].

AIM OF WORK

Aim of the work is to detect the role of PET/CT in evaluation of patients after surgical excision of colorectal cancer to detect any recurrence or metastatic lesions.

MATERIALS AND METHODS

From December 2018 through December 2019, 20 patients 12 males and 8 females with a history of operated colorectal cancer and suspected local recurrence at operative bed or distant deposits were included in the study. The age of the patients ranged from 43 to 64 years with mean age was 56±5.95. The inclusion criteria were as follows: patients with cancer colon or rectum who underwent surgical resection and those with suspected clinical or laboratory recurrence or metastasis. Serum tumor markers of CEA and enhanced CT were performed for all patients before PET/CT scanning. Patients with the following conditions were excluded from the study: serum creatinine level above 2mg/dl, recent surgery within less than 4 weeks, radiotherapy within less than 3 months, chemotherapy within less than 4 weeks, pregnant females and those with sensitivity to iodinated-contrast agents.

Ethical consideration

The study was done according the Code of Ethics of The World Medical Association (Declertion of Helsinki) for studies involving humans

Institutional Review Board (IRB) of Zagazig University confirmed our protocol and consent forms. A written informed consent

was signed by all participants about demographic and clinical data.

CT technique

Multi-detector CT scanning was carried out on all patients included in the study. (GE-DISCOVERY) according to an established protocol. After 6 hours fasting, in a supine position, each patient underwent full-body enhanced CT from the base of the skull to the upper thighs. A total amount of 100 -150 ml of iodinated contrast material was administered intravenously through power injection at a rate of 2.5 ml/s, and After injection, the scan was performed at 140 kV and 120 mA with 4 mm section collimation, 13.5 mm table speed per rotation and 3-7 mm reconstruction thickness. Using a soft-tissue algorithm, the transverse images were reconstructed. No oral contrast agent was given.

PET/CT scanning

18F-FDG PET/CT scans were done using an integrated PET/CT system (GE DISCOVERY) at military hospital. After 6 hours fasting except of water, in all patients prior to FDG injection, blood glucose levels were measured and no patients displayed a blood glucose level higher than 160 mg/dl then all patients have been injected 4.4–6.8 MBq/Kg of 18Ffludeoxyglucose (18F-FDG). An intravenous injection was administered into the elbow vein using a previously implanted infusion line. After injection patients were asked to rest in a quiet worm waiting room and avoid any stressful or muscular activity for at least 60 min. slice helical CT acquisition is the first step used in the PET/CT system, then a full-ring dedicated PET scan of the previously examined axial region. X-ray tube voltage peak of 120 keV, 90 mA, a 5:1 pitch, a rotational speed of 0.8 s/rotation and slice thickness of 5 mm, used as the CT element, while PET elements were done from head to thigh with 4 min per field of view, each attempting to cover 14.5 cm, and axial sampling thickness of 4.25 mm/slice. Both scans were done while the patients were normally breathing. With the help of using ordered-subset expectation software PET cuts were reformatted with CT derived attenuation correction. The results as the

attenuated corrected PET images, CT images, and the fused PET/CT images with reformatting in different plans (axial-coronal-sagittal) were obtainable. The attenuation-corrected PET images, CT images, and fused PET/CT images were available for being reviewed in axial, coronal and sagittal planes.

Analysis on imaging and data

Contrast-enhanced CT images, have been assessed and analyzed retrospectively in different sections (axial, coronal and sagittal) with proper window setting for different lesion identification. Detection of heterogeneously enhanced soft tissue mass of different sizes and shapes in the peritoneal surface or even implanted in peritoneal fat were diagnosed as peritoneal metastasis and infiltration. Malignant Lymph nodes (LNs) were diagnosed when had short axis larger than 1 cm or with central necrosis in the form of central low enhanced area, while presence fatty hilum of the lymph node was a benign sign regardless of the actual nodal size [6–8]. The CT, PET and fused PET/CT Images have been simultaneously opened and analyzed using visual observation and semi-quantity analysis by a team of nuclear medicine doctors and radiologists evaluating and reviewing PET images and CT images. Semi-quantity analysis by measuring the maximum standard uptake value (SUV max) of the region of interest, if exceeding 2.5 should be considered positive uptake region for malignancy, however some benign inflammatory pathologies of the intestine had high uptake and recorded (SUVmax) reaching 5 that considered a great pitfall of the exam, except of the later showed no definite space occupying lesions or definite diagnostic criteria on the combined CT scan [13]. histopathological biopsy of the accessible lesions and comparing the findings with clinical follow up, other methods of diagnosis as (tumor markers: CEA) and different imaging modalities used as the gold standard in our study for confirmation of recurrence of metastasis detection.

Statistical analysis

Results were statistically informed in terms of range, mean \pm standard deviation (\pm SD),

median, frequencies (number of cases) and percentages if appropriate. Kruskal Wallis analysis of variance (ANOVA) test used for comparing quantitative variables between different categories of our study groups, Chi square (χ^2) test was used as well. P values less than 0.05 was recorded as statistically significant. All statistical results were done through computer programs Microsoft Excel 2017 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

RESULTS

Twenty postoperative patients with surgically excised colorectal carcinoma underwent whole body FDG PET/CT for follow up with the final histopathological and formal clinical follow-up findings were referred as gold standard to compare between the sensitivity and specificity of FDG PET/CT and CECT of the same time.

On patient-based distribution; recurrence was detected in 9 (45%) patients by CECT and 12 (60%) patients had metastatic lesions. While PET/CT detected recurrence in 10 (50%) patients and metastatic lesions in 15 (75%) patients. On the other hand according to site-based analysis CECT detected 9 recurrent lesions and 36 metastatic lesions at different organs as liver 5 (13.8%), spleen 2 (5.5%), bone 7 (19.4%), lung 6 (16.6%), peritoneal 5 (13.8%) and L. Ns 11 (30.5%). while PET/CT detected 10 recurrent lesions and 48 metastatic lesions at different organs as liver 9 (18.45%), spleen 2 (4.1%), bone 10 (20.8%), brain 1 (2%), lung 6 (12.5%), peritoneal 7 (14.5%) and L. Ns 13 (27%). In our study we detected 1 recurrent lesion and 12 metastatic lesions by PET/CT that were missed by CECT that led to restaging and changing the line of treatment of the patients as it depends on cellular metabolism rather than morphological changes table (1).

Regarding the gold standard distribution CEA was performed to all patients, elevated CEA level was found in 18 patients, histopathological biopsies were taken from accessible lesions in 10 patients and MRI was performed for 8 patients.

Histopathological analysis of lesions showed that adenocarcinoma was the most common type among all participants about 9 patients (81%) while 1 patient (9%) was mucinous adenocarcinoma. A suspected lesion was reported as true-positive (TP) if the histopathological biopsy was positive or if the lesion displayed any progression at 6 months of follow-up imaging; PET/CT detected 9 TP lesions and CT detected 8 TP lesions. A lesion was reported as true-negative (TN) if the histologic report were negative or the lesion vanished or was found unchanged at follow-up imaging and without clinical decline for at least 6 months; PET/CT detected 9 TN lesions while CT detected 8. The lesion was considered false positive (FP) if histopathological biopsy of suggestive lesions were negative for carcinoma or the lesions had disappeared on subsequent follow-up imaging; both PET/CT and CT detected 1 FP. Lesions were false-negative (FN) if the findings of CT or PET/CT were negative but on clinical follow up there is persistent increase or rising CEA levels or if disease progression was seen on other imaging modalities; 1 lesion was considered FN by PET/CT and 3 lesions were detected by CT Table (2).

Statistical analysis of the recurrence results showed that PET/CT has high sensitivity 90%, specificity 90%, PPV 90%, NPV 90% and accuracy 90%. While CECT showed 72.7% sensitivity, 88.8% specificity, 88.8% PPV, 72.7% NPV and 80% accuracy.

Regarding metastatic evaluation; CECT was able to detect metastatic lesions on a patient-based analysis with a sensitivity 68.7%, specificity 75%, PPV 91.6%, NPV 37.5% and diagnostic accuracy 70%, while PET/CT had a sensitivity 87.5%, specificity 75%, PPV 93.35, NPV 60% and diagnostic accuracy 85%.

On a site-based analysis, CECT had a sensitivity, specificity and accuracy of (68.1%, 63.6% and 67.2% respectively), with a PPV 88.8% and NPV 31.8%, while PET/CT had a sensitivity, specificity and accuracy of (95.7%, 72.7% and 91.3% respectively), with a PPV 93.7% and NPV 80% table (3).

Table 1: Findings by CECT and PET/CT Patients and Site- Based Distribution:

		CECT		PET/CT	
		Patient (n=20)	%	Patient(n=20)	%
Recurrence		9	45	10	50
Metastasis		12	60	15	75
		Lesions(n=36)	%	Lesions (n=48)	%
	Brain	0	0	1	2
	Bone	8	22.2	10	20.8
	Liver	5	13.8	9	18.75
	L. Ns	11	30.5	13	27
	Peritoneal	5	13.8	7	14.5
	Lung	6	16.6	6	12.5
	Spleen	1	3.6	2	4.1

Table 2: Association and agreement between gold standard, PET CT and CECT regarding metastasis and recurrence detection

Metastasis (patient-based)			Gold standard		Total	X ²	P-value
			-VE	+VE			
	PET/CT	-VE	3	2	5	5.65	0.021*
		+VE	1	14			
	CT	-VE	3	5	8	4.18	0.043*
		+VE	1	11			
Total			4	16	20		
Metastasis (site-based)			Gold standard		Total	X ²	P-value
			-VE	+VE			
	PET/CT	-VE	8	2	10	13.22	0.0004*
		+VE	3	45			
	CT	-VE	7	15	22	3.98	0.049*
		+VE	4	32			
Total			11	47	58		
Recurrence			Gold standard		Total	X ²	P-value
			-VE	+VE			
	PET/CT	-VE	9	1	10	12.8	0.00*
		+VE	1	9			
	CECT	-VE	8	3	11	8.58	0.00*
		+VE	1	8			
Total			10	10	20		

Table 3: validity of both CECT and PET/CT in detection of recurrence and metastatic lesions:

		Sensitivity	Specificity	PPV	NPV	Accuracy
Site-based metastatic lesions	PET/CT	95.7%	72.7%	93.7%	80.0%	91.3%
	CECT	68.1%	63.6%	88.8%	31.8%	67.2%
	<i>P-value</i>	0.031*	0.43	0.71	0.00**	0.013*
Patient-based metastatic lesions	PET CT	87.5%	75%	93.3%	60 %	85%
	CT	68.7%	75%	91.6%	37.5%	70 %
	<i>P-value</i>	0.64	-----	0.94	0.34	0.61
Recurrence	PET CT	90.0%	90.0%	90.0%	90.0%	90.0%
	CT	72.7%	88.8%	88.8%	72.7%	80.0%
	<i>P-value</i>	0.64	0.44	0.48	0.87	0.74

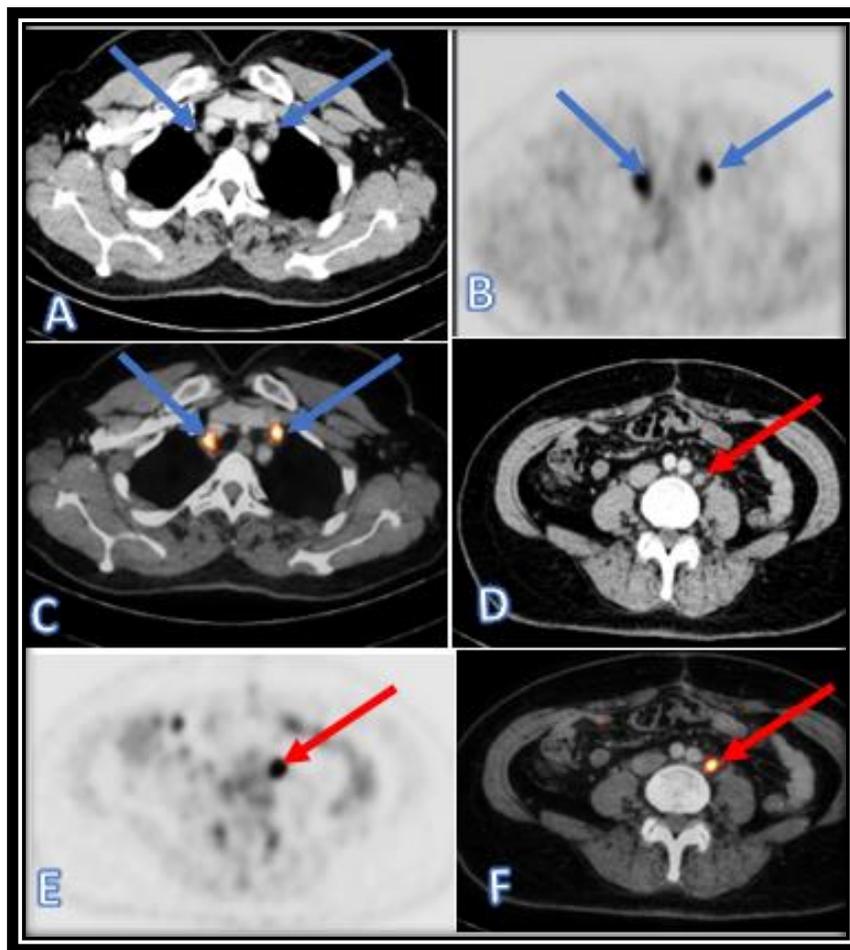


Figure (1): A 48-years old female patient, presented with history of colonic cancer that was surgically resected followed by chemotherapy, presented now with increasing elevated CEA serum level, on CECT no suspicious lesion was detected (A): Axial CECT image shows bilateral deep cervical L.Ns that considered non-malignant according to CT criteria (normal size and rounded shape) (B): Axial FDG PET image shows bilateral metabolically active 18F-FDG avid deep cervical L.Ns (blue arrows) with SUV achieving (6.1) (C): Axial fused PET/CT image confirmed the location of the bilateral metabolically active 18F-FDG avid deep cervical L.Ns (blue arrows) (D): Axial CECT image shows a solitary left common iliac L. Ns (red arrow) that considered non-malignant according to CT criteria (normal size and rounded shape). (E): axial FDG PET images shows a solitary metabolically active 18F-FDG avid pelvic L.N (red arrow) with SUV max achieving (8.2) (F): Axial fused PET/CT image confirmed the location of the metabolically active 18F-FDG avid left common iliac L. N (red arrow).

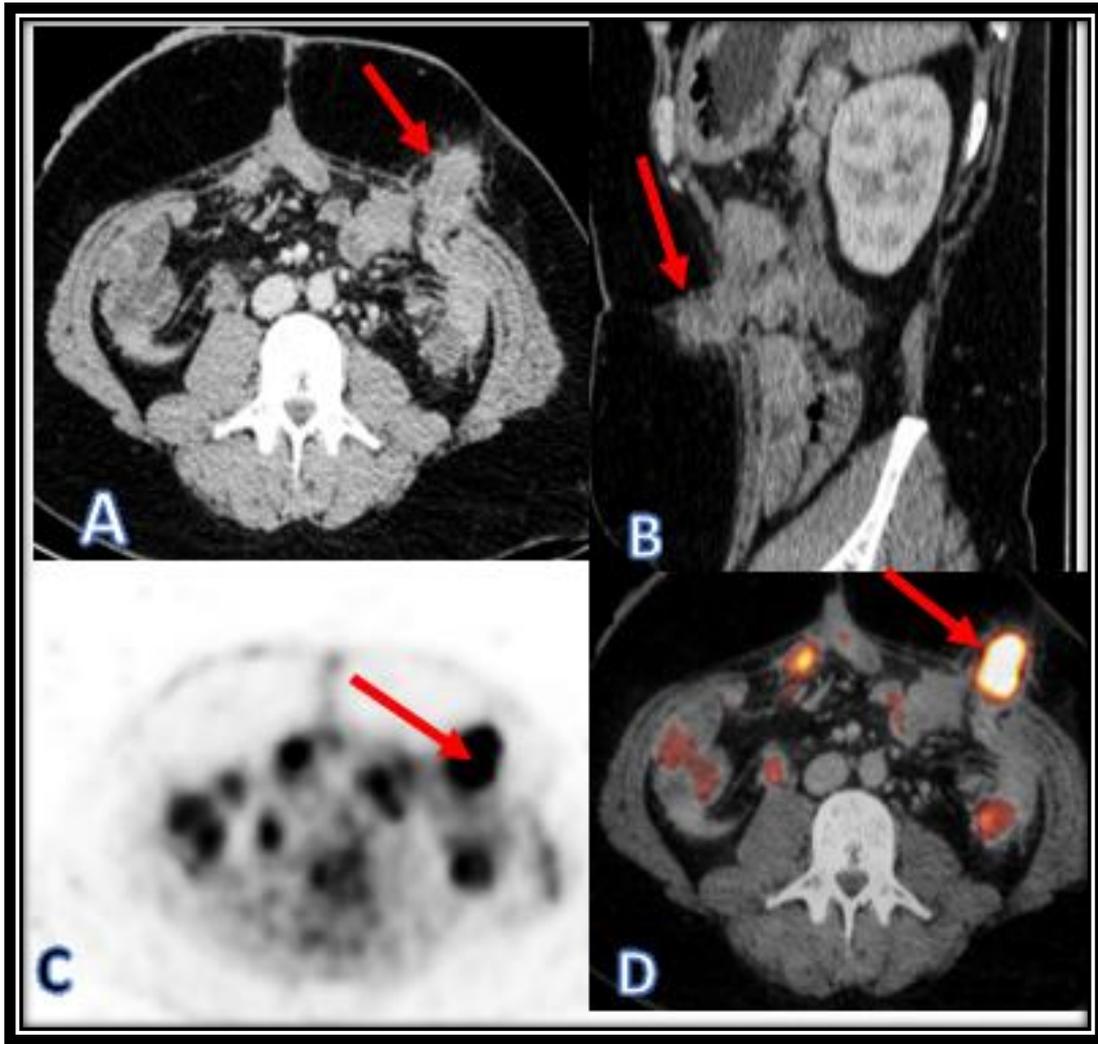


Figure (2): A 47-year-old man with history of cancer colon and left hemi-colectomy 9 months ago, complained from subcutaneous palpable mass at the left lumbar region. **(A):** Axial CECT and **(B):** sagittal CECT image shows an ill-defined omental mass at the left lumbar subcutaneous region (red arrow) measuring (4x3x3.5 cm), with internal enhancement. **(C):** Axial FDG PET image shows a metabolically active ^{18}F -FDG avid mass seen at the left lumbar region (red arrow) achieving SUV (9) **(D):** Axial fused PET/CT image confirmed the location of the metabolically active ^{18}F -FDG avid mass at left lumbar subcutaneous region (red arrow).



Figure (3): 65-year-old male patient operated colorectal cancer 1year ago, presented by elevated CEA and suspected recurrence but no recurrent or metastatic lesions were detected on follow up imaging modalities PET/CT was recommended for metastatic work up (A): Axial CECT bone window at the level of C7 cervical vertebrae shows no abnormal osteolytic or sclerotic bony lesion. (B): Axial FDG PET image shows a small metabolically active 18F-FDG avid lesion seen at the right vertebral pedicle of cervical vertebrae C7 (red arrow) achieving SUV_{max} (4.5) (C): Axial fused PET/CT image confirmed the location of the metabolically active 18F-FDG avid lesion at the right vertebral pedicle of cervical vertebrae C7 (red arrow).

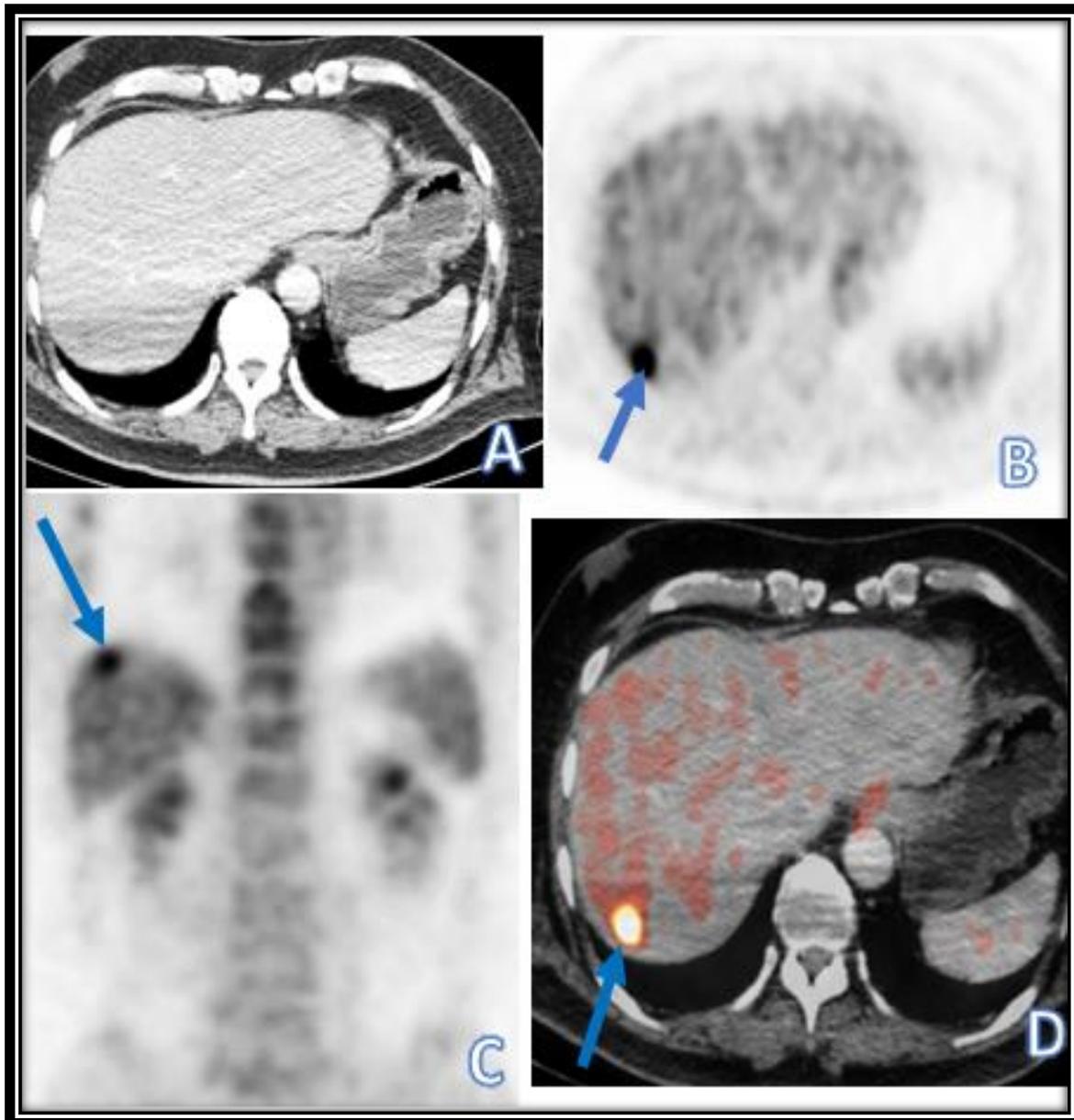


Fig (4) A 56-year-old man with history of operated colorectal cancer followed by chemotherapy referred for follow up. **(A)**: Axial CECT of the abdomen shows no hepatic deposits or other metastatic lesions. **(B)** Axial FDG PET image, **(C)** coronal FDG PET image shows a solitary hepatic metabolically active FDG avid focal lesion (arrow) that achieving 7.5 SUV_{max} . **(D)** Fused PET/CT at the abdomen confirmed the location of the metabolically active hepatic focal deposit at right hepatic lobe (segments VII) (arrow).

DISCUSSION

As CRC has a very high recurrence rate and mortality rate, its crucial to use an effective imaging modality for staging, follow up and post-operative evaluation [2]. For surgeons and oncologists CECT used to be the best among multiple imaging modalities for follow up operated colorectal cancer and assessing

the respectability of recurrent tumor lesions or residuals, also for further neo-adjuvant chemotherapy, immunotherapy or radiotherapy [4]. Until then, the actual tumor burden could be greatly underestimated. Depending on size criteria and anatomic changes limits the reliability of CECT in the diagnosis of malignancy at operative bed [13].

Though the malignant mass more likely to be larger on size, some benign fibrous tissue tends to be large with architectural distortion around as a result from post-therapeutic inflammatory changes (post chemotherapy and radiotherapy). CECT has specificity issues and inability to differentiate viable from nonviable tumor lesion [5-7].

On the other hands Combined PET/CT has already shown its superiority over PET or CT alone in restaging of patients with operated colorectal cancer [14]. Whole-body PET/CT can detect metastasis in all body parts at one exam at the same time, which is important for choosing treatment regimen, especially to avoid unnecessary reoperation even before morphological changes occur, because PET/CT depends on cellular glucose metabolism of the lesions not only morphological changes that may take longer time to occur [15].

PET/CT also has a great role appears in detection of small sized LNs, small suprarenal metastasis, early osseous deposits and post-therapeutic inflammatory changes [16]. But PET/CT also has imitations includes its inability to detect viability in tiny small subcentimetric hepatic focal lesions and small pulmonary nodules as well as the detection of mucinous tumor lesions with abundant mucin, recently using delayed regional scan is much better to detect such lesions [8-10].

Up to 6 months after surgery post-operative inflammatory changes still seen at operative bed. So, it's better to perform the scan at least 3 months after surgical excision [12], in this study it was mandatory to perform the exam at least 6 months after initial surgery to avoid such false positive results .

In our study we included 20 patients 12 males and 8 were females with a history of operated colorectal cancer and suspected local recurrence at operative bed or distant deposits mean age of study participants was 56 ± 5.95 years, median age was 58 years, minimum age was 43 and maximum age was 64 years. Clinical information, follow-up and pathological reports of the patients were reviewed as the gold standard of our study.

Histopathological analysis of the recurrent and metastatic lesions of our study showed that adenocarcinoma was the most common type among all participants about (81%) while (9%) were mucinous adenocarcinoma that matches the results of the study by Lee et al [15] and our results are in concordance with the study done by Pen et al [16] that proved the high sensitivity and specificity of PET/CT in detection of recurrent CRC and changed the planned management of 48.4% (62/128) of all patients. Another study by Gade et al [17] that agreed with our results showed the role of PET/CT in detection of recurrent CRC among 35 patients with high sensitivity 85.7% ,specificity 94.7% ,PPV 93.8 and NPV 87.8 %.

Zidan et al [18], compared the ability of PET/CT and CECT in detecting recurrent lesions studied on 7 different 1^{ry} malignant tumors (One of them was colorectal cancer) for postoperative restaging of 42 patients with suspected recurrence results from new clinical, biochemical and radiologic findings were prospectively evaluated, PET/CT results changed the management of 90% of patients. A final diagnosis of recurrence was confirmed by biopsy or by further clinical and radiologic work-up. For the site-based analysis PET/CT showed 100% sensitivity, 80% specificity, 98% PPV, 100% NPV and 98% diagnostic accuracy compared with 87%, 50%, 94%, 28% and 83%, respectively, for CT. For the patient-based analysis, PET/CT showed 100% sensitivity, 75% specificity, 97% PPV, 100% NPV and 98% accuracy compared with 86%, 75%, 97%, 38% and 86%, respectively for CT.

Cha et al [19] also confirmed that the advantages of PET/CT for the restaging are mostly refers to the detection of pathological L. Ns with high uptake and normal size, that matches with our study in detection of 2 pathological groups of L. Ns by PET/CT that misdiagnosed by CECT Fig (1).

In another study by Hussein and Nassef [20], comparing between PET/CT and CECT in evaluation of postoperative recurrence and metastasis in 96 of colorectal cancer patients; the specificity of PET/CT (67.4%) was statistically significantly better than that of

CECT (30%) as it can reduce the false positive results of CECT in 13 patients, even so the sensitivity PET/CT (88.3%) displayed higher value than CECT (77.3%) but without statistical significance proving that PET/CT is much better as follow up imaging modality.

Another cohort study by Fehr et al [21] that supports our study findings was performed on 50 patients with operated CRC stage III and negative metastatic lesions by conventional preoperative imaging modalities, the postoperative PET/CT results changed the management strategy of 7 patients (14%) who were proved to have misdiagnosed metastatic lesion at liver, lung, bone, peritoneal and L.Ns before starting the neoadjuvant therapy.

Lee et al [15] had performed both PET/CT and multidetector CT (MDCT) on 266 patients with colorectal cancer to assess the value of PET/CT over MDCT in the staging of colorectal cancer. MDCT and PET/CT showed similar accuracy in detecting lymph node metastasis in patients with clinical stage III and stage IV disease. PET/CT led to a change in treatment protocol for 1 of 40 patients (2.5%) with clinical stage I, 9 of 138 patients (6.5%) with stage III, and 8 of 63 patients (12.7%) with stage IV disease. But had no role in stage II diseased patients. Such results with high sensitivity and specificity of PET/CT for staging colorectal cancer patients matches with our study making PET/CT better than CECT as imaging modality in CRC patients.

On the other hand study by Paspulati et al [22] comparing between the role of PET/MRI and PET/CT in staging and restaging of colorectal cancer on 12 patients their results proved that PET/MRI is better in staging of tumor invasion of tissue (T staging) than PET/CT as it provides more details about soft tissue contrast, similar results in N and M staging between both modalities, however these results can't be generalized as the small sample size of the study.

In conclusion, PET/CT is a very good imaging method for evaluating post-operative colorectal cancer patients whom suspected to have recurrent or metastatic lesions, with higher sensitivity and specificity than the CECT, that leads to restaging and changing

therapy protocols and decreasing the risk of unnecessary surgeries.

The authors declare no conflict of interest.

No financial or personal relationships with other people or organizations relevant to the subject of the manuscript that could inappropriately influence the authors' actions.

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