The Libyan Journal of Science
University of Tripoli Vol. 26, No. 01
https://uot.ed.ly/journals/index.php/ljs

Fractal Analysis on the Detection of the Malignancy Changes of Pancreatic Cancer

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ARTICLE INFO

Article history:
Received 09/01/2023
Received in revised form 21/05/2023
Accepted 22/05/2023

ABSTRACT

Carcinoma of the pancreas is frequently associated with spatial changes between the centre and periphery of tumours. In order to convert histopathological objectives into new prognostic criteria for clinical needs, a semi-automated approach is investigated using fractal. Three different segments, known as the lumen, cell cytoplasem, and stromal tissue, of pancreatic malignancy were considered under this approach. Method concerning tissue preparation, morphological-segmentation, and applied parametric extraction were applied for examination and factor identification. For this purpose, samples from 21 cases of pancreatic carcinoma were stained, using the sirius red, light-green method. A number of 105 samples from each location (center/periphery) were obtained for examination as images in 512x512x3 pixel format. Segmentation was achieved using a clustering technique, and images were manually segmented into representative colors. For feature extraction, masked images were used and pre-processing approach of hue, saturation, and intensity color space was applied. Estimated fractal dimension were analyzed to examine whether the self-similarity features can be usefully implemented to detect changes in the tumour invasion between and within individual groups. Obtained results showed that fractal dimension is found on the malignancy of pancreases. This work supports scopes for the advantage of using automatic geometric analysis to define diagnostic markers on pancreatic tumour.

Keywords: Carcinoma; cell cytoplasem; malignancy; histopathology

1. Introduction

Pancreatic cancer remains the seventh leading cause of cancer-related deaths worldwide (4.7% of the total cancer deaths). Pancreatic cancer remains one of the most lethal malignant neoplasms that caused 466,003new deaths in 2020 (GLOBOCAN 2020 estimates). Globally, 495,773new cases of pancreatic cancer have been reported in 2020,[1,2]. To date, the causes of pancreatic carcinoma are still insufficiently known. Despite developments in detection and management of pancreatic cancer, the 5-year survival rate still stands at only 9%. It is still a highly lethal cancer with a low 5-year survival rate. Therefore, the 5-year survival rate of pancreatic cancer varies globally in different regions and

countries, but does not exceed 10% [3,4]. During the period 1996-1999, pancreatic cancer had the lowest relative survival rate among the 15 most commonly diagnosed cancers in both genders, with only less than 3% of patients surviving five years. To date, the causes of pancreatic carcinoma are still insufficiently known and we need to understand the biological mechanisms that contribute to development and progression of pancreatic tumours. Recent reports announced that more research is essential to understand cancer progression for therapeutic purposes [3,5].

1.1. Quantitative analyses in pancreatic histology

Pancreatic cancer is a malignant tumour with an extremely poor prognosis. Several prognosis and diagnosis analysis approaches based on morphology, and stereology of histological dataset were reported [6,7]. However, the use of quantitative analysis in the histology of the pancreas is still limited as most biopsy samples are still assessed manually.

Manual methods are time consuming and subject to the sampling error. Human vision is qualitative and comparative but not quantitative. It judges the relative size and shape of objects by mentally manipulating them to the same orientation and overlapping them to accomplish a comparison. This visual manipulation affects samples evaluation and afterwards decision-making. Thus, quantitative analysis in histology becomes essential. A comparison between manual and semi-automated image analysis of pancreatic cancer was reported [8,9,10,11]. Semi-automated image analysis achieved better results, both in measuring tissue area fraction and in repeatability, than manual methods which based on point counting. More points are required to achieve the same repeatability as for the semi-automated technique pointing the advantage of quantitative analysis of histological measurements. Evaluated microvasculature by investigating the micro-vessel, of pancreatic cancer, under a light microscope and by image analysis. Their estimated parameters were highly correlated, and they showed that automated image analysis offered new parameters with some predictive value which might help in clinic management. The image analysis could provide useful information to discriminate adenocarcinomas arising from the bile ducts or ampulla from those arising in the pancreas [12, 13]. An imaging modality that provides higher tumor detectability would be desirable, morphological heterogeneity reflects structural and functional diversity in key cancer biological processes. The image analysis based morphology could detect spatial changes in the histopathology of pancreatic tumours. More stromal area of 10.0% points average were found at the periphery of the tumours than its centers, which was associated with tumour reaction on the fibrous tissues of host glands [14, 15, 16]. Wolnicka et al [17] used morphological approach based on the shape to distinguish between cancerous and normal nuclei in adenocarcinoma of the pancreas. Four morphological features were obtained suggesting combining these features for classification analysis. Okon et al [18] used texture and geometric parameters to classify grey level ductal pancreatic carcinoma. A correct classification of 73% was achieved using artificial neural network for early diagnosis of ductal pancreatic carcinoma.

1.2. Stroma tissue interference

Histological specimens obtained from the head of the pancreas for investigation were pathologically treated to produce stained microscopic slides. Pancreatic tumour tissues consisted of three different tissues known as the cell cytoplasem, lumen, and stromal tissue, (fig (1). Due to the cancer, an intense growth of fibrous tissue can be detected at the periphery of tumours. Due to the recent information limit, the finding of [14-19] suggested further quantitative measurements investigation to be required to identify new prognostic factors. Recent pancreatic tumour biology research has focused upon the stromal reaction. There is an indication that its histopathological quantification might lead to produce novel prognostic markers. Such target is very important as it could explain extents of the tumour invasion and metastasis that leads to determining pancreatic tumour response to conventional and novel therapies. Sohn [19] in his review has also pointed to the association between the genetic mutations and syndromes with pancreatic ductal carcinoma.

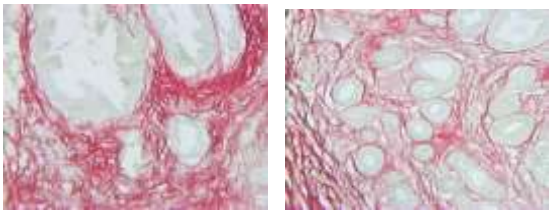

Fig. 1. Examples of Sample Images of Pancreatic Tumours at different positions. Centre (up), and at the Periphery (down)

1.3. Digital image processing

Digital image processing is one of the fastest growing application fields in science and engineering [20, 21]. Image analysis techniques were proposed to examine the histological aspects of pancreas cancer. Several digital image-processing techniques such as segmentation and feature extraction of the true color RGB images are required for this work. According to recent advances on dealing with similar problems on 2D histological medical images[22], an image description should be carefully considered. A diagram of proposed image processing operations can be seen in figure (2). Recent mathematical morphology advances were reported[23] to facilitate segmentation and feature extraction. Marghani et al [22] demonstrated that abnormalities in low-level power could be distinguished using quantitative measurements based morphology. Whereas, several researches have used different approaches based upon quantitative morphology, and stereology on the examination of abnormalities on pancreas cancer, but less attention was paid to assess tumour progressive of pancreas. Sims et al[14] showed that special changes on fraction area of pancreatic cancer could be detected between the centre and periphery of the same tumour. Stromal component was more likely to be effected by cancer, which exhibited by its infiltration on fibrous tissue. Therefore, more stromal tissue at the periphery than the centre was detected [14]. This suggests that self-similarity property of using fractal dimension could be found in pancreas cancer. Further shape factors parameters based on the area and perimeter, which has been examined [22], might be useful to consider later.

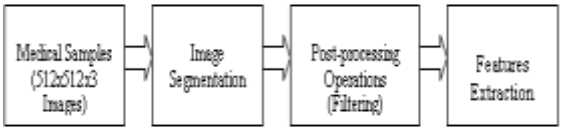

Fig. 2. The diagram show applied image-processing process

The quantitative image analysis approaches need investigation to identify special differences in the histopathology of pancreatic tumours. Stromal tissue reaction had exhibited changes on area fraction between periphery and the centre of the pancreatic tumour, and therefore, we expected that an investigation using geometric features could produce valuable prognostic information within and between tumour dataset. Furthermore, assuming HSI color space could be useful to examine color attribute on image interpretation in several domains.

2. Materials and methods

Pancreatic tumours dataset supplied for this research were collocated over a period of thirteen years ending on 1996 under similar treatment using Kausch-Whipples pancreatic duodenectomy. A number of 21 cases, came form the head and ampulla of Vater of the pancreas tumours, were randomly chosen. Biopsies obtained from the head of the pancreas consisted of four different tumour organs, known as cholangio carcinoma n=2, ductal carcinoma n=3, and n=5 uncertain class within the same field. Samples were received fresh and fixed in formalin for a period of 24-hour. The head of pancreas was then serially sliced in parallel to the common bile duct to produce sample blocks. Using sirius red, light-green method[24], all samples were then pathologically stained. Every single tissue type can be recognised by specific color, where shades of red color represent stromal tissue, and the shades of green indicates epithelium tissue, while the clear spaces corresponds to ductal lumina.

Using a Leica Q500 MC image analyzer system, images were taken at a single magnification (objectives x 4) containing an area of 8.64 mm^2 for analysis per frame. Images were stored on 512 x 512 x 3 image size format and each element occupied 8 bits. Five neighboring images were taken from the centre and the same at the periphery from each cancer slides producing an outcome of 105 images at each position. Image selection is done by a specialist pathologist, Freeman hospital (UK). Centre neighbored images were taken from the nearest areas to the centre of gravity of the tumor, whilst periphery samples were captured as close as possible to the infiltrative margins. Examples of the final outcome were presented on fig. (1).

2.1. Automatic segmentation of color images

Segmentation is a process that divides the images into regions that are homogeneous with respect to local attribute such as, intensity and color. Stained images obtained from the histological samples of pancreatic tumours were treated by sirius red, light-green method, which produce color images containing special information of each tissue components (fig (3)). In order to detect valuable morphological information from each tissue component, each image was segmented and

producing three RGB masked images representing the tissue components. Each tissue type appears on different color range information produced by staining reaction. Due to the advantage of HSI color

domain of being more sensitive to color characteristic which is similar to the process humans manipulate images [20, 25], and therefore, processed masked images were studied under H-domain, S-domain, and I-domain separately. Segmented images were then subject to a feature extraction process in which many measurements based of shape and size were estimated at each single tumour position.

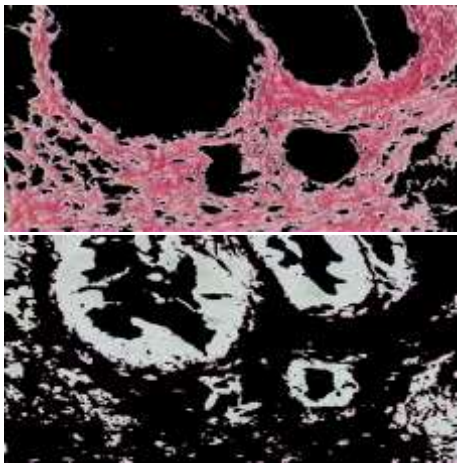

Fig. 3. Masked Images of Cell Cytoplasem (up) and Stromal Tissue (down) in 24-bit format

2.2. Geometric measurements

The use of geometric measurements on histological analysis of carcinoma was reported [6,7].

In this work, the method employed were based on property of self-similarity that give quantitative description to the structure, and in general, provide measurements of the geometric cells and tissues. Fractal dimension based on self-similarity property where also considered to quantify roughness objects using box-counting method to configure fibrous tissue of pancreatic cancer. .

$$FD = \lim_{L \to 0} \frac{\log N(L)}{\log (1/L)}$$

where L is the box width, and *N (L)* is the number of boxes (*N*) needed to cover a surface.

2.3.Statistical analysis

Within- and between-tumour variation of specific tissue parameter

Cracinoma of pancreas exhibits differences in the size of enlarged glands within tumours, which comes from the same image frame dimension. Each set of individual samples is usually depended on the positioning of the frame with respect to the local glandular structure. This produced a frame-to-frame variations associated with size, and therefore it was necessory to take the parameter average for each set of frames at each location (centre, periphery) for each tumour. However, the relative size of each tissue type can also be seen in fig (3). For all parameters, variation

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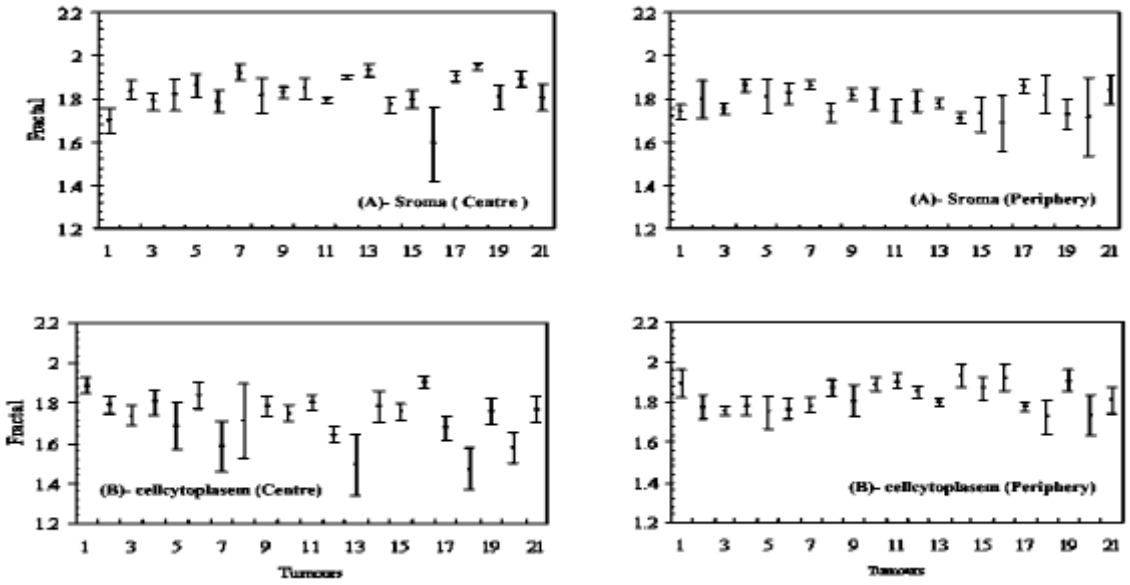

Fig. 4. Fractal dimension parameter (Mean ± Standard deviation) of stroma (A), and cellcytoplasem (B) at the centre, and periphery, of 21 tumours. Each point corresponds to five observations of the represented tumour within-tumours and differences between-tumours were estimated using analysis of variance

2.4.Detection of overall spatial differences

At each tumour location, mean values of the extracted parameters of the five samples were estimated. The mean differences between centre and periphery for the 21 tumour samples were also calculated. Thus, initial information was derived as to whether ot not there was an overall spatial difference, using the estimated factors, within tumours of 21 cases of the cell cytoplasem and stroma tissues. The basic statistics of mean, standard deviation, and skew of mean differences were all measured, and finally the confidence interval (CI) at 95% significant level that the mean differences was away from zero were estimated using student t-test.

2.5.Detection of spatial differences within individual tumours

Using a method presented for calculating the confidence interval for the difference between the two means[26], the ability of detecting spatial differences by each single parameter on each tissue type were calculated. This were achived by subtracting the means of (centre, periphery) values of 21 tumours for each parameter investigated. For all measurements; samples variances of the two location groups were assumed to be equal (pooled t-test); the confidence interval at the 95% significance level was measured for tumurs, and the results showed a significant difference between the means of each two populations of the five independent samples.

3. Results & Analysis

In order to quantify the morphological information that based on the shape and structure of the object tissues, geometric approaches were investigated. Segmentation was directly employed to the masked images. Therefore, each image pixel assumes only one of two discrete values, belonging to the background objects and the glands objects of specified tissue components. A set of measurements described from those whose described on section were estimated for the stroma, Table 1(a), and cell cytoplasem, part (b), tissues of pancreas cancer. Semi-automated image analysis show histological changes in tumours of the pancreas cancer can be identified using fractal. Significant result at 95% levels of up to $P<0.0001$ in the detection of special differences between the centre and the periphery of pancreas cancer were achieved.

Table 1. Obtained results, a set of fractal dimension of stroma component (A), and cell cytoplasm components (B), at the centre and periphery of 21 tumours. Mean feature value for each estimated parameter was calculated from five samples taken from both regions of each tumour. Variations within-and between-tumour of 21 sets of five samples at location, centre and periphery, were calculated by analysis of variance. The P-values were also presented to show the ration of between-to within tumour variances

Variability	Stroma		Cell-Cytoplasem	
	Centre	Periphery	Centre	Periphery
Mean	1.723743	1.82321	1.8265	1.7808
Maximum value	1.9452	1.9887	1.9854	1.9301
Minimum value	1.2557	1.6353	1.4359	1.4418
Between tumour (SD)	1.3604	0.4372	0.6561	0.2909
Within tumour (SD)	0.5868	0.2866	0.2873	0.4166
P-Value	<0.0001	<0.0001	<0.0001	<0.0001

Table 2. Overall special fractal changes of (A. Stroma tissue component, and B. Cell cytoplasem component) using a set of morphological features were presented. The evaluation were presented as the means difference between (centre, periphery) of 21 tumours. Confidence interval at 95% was calculated with the probability that the observed difference arose, caused by chance

Variability	Stroma		Cell-Cytoplasem	
Mean difference	0.099467		-0.04541	
Standard deviation	0.098747		0.067542	
Conf. Interval at 95%	0.0545	0.1444	-0.0762	-0.0147
P-value	0.0001		0.0059	

3.1.Within-and between-tumour variation of tissue parameter

The results showed that, the tumours variation of each tissue component is larger than the within-tumour variation. Figure (4) show the fractal dimension for 21 tumours, numbered chronologically, of stroma and cell cytoplasem tissue components at the centre and periphery. Using analysis of variance, this finding is also confirmed in ($P<0.0001$) that the variability within tumour samples was smaller than between tumours, where each tumour sample processed using five observations at each location.

3.2.Detection of over all special differences

As shown in Table 1, and Table 2, results obtained from stroma tissue component, all features show clear differences between the periphery and the centre over 21 tumour cases. Higher significant differences of ($P<0.0005$) were detected using the fractal dimension. In contrast, less significance on cell cytoplasem tissue of ($P<0.01$) using the FD were defined on the differences between locations (periphery, Centre). The difference this time was on the opposite direction to the one obtained on the stroma tissue

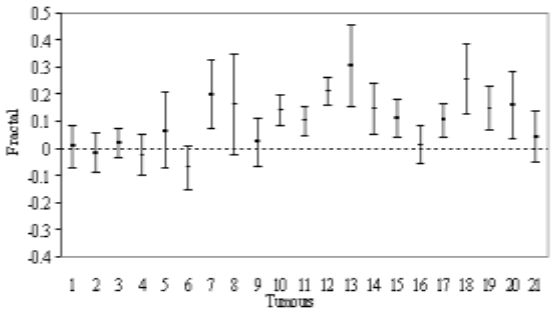

Fig. 5. Show FD with error bars at 95% confidence intervals of the mean dif. using the stroma tissue.

3.3. Detection of special differences within individual tumours

As it can be seen in fig(5), which show the most significant change found, the fractal able to detect tumour difference in up to 11 cases. Those cases show a significance increase ($P<0.005x10^{-3}$) on the stroma roughness of stroma tissue at the periphery than that at the centre with mean increase in stromal roughness of 0.1701± 0.0624 points at 95% significant difference. At 95% Confidence Interval the Table (2) shows that both tissues shows clear behavior above or below the zero.

4. Conclusion

Automated digital image processing showed valuable information can be detected from histological images using geometric analysis. Fractal was found in pancreas cancer and more roughness was detected at the periphery of stroma tissue component. In contrast, FD at the centre of the tumour of cytoplasem tissue is higher than the periphery. In general, using morphology the estimated measurements show clear differences between the centre and periphery for all cases. Variations within tumours are smaller than between tumours of 21 cases at the periphery and the centre of pancreas cancer, and ($P<0.001$) were achieved using analysis of variance. There are only six cases that showed no special differences associated to the tumour reaction. In agreement with similar work the system is able to detect differences on 11 cases. Our results show tumour reaction can be defined in both stroma, and cytoplasem tissue components. Further analysis to the texture structure of pancreas cancer can also be useful to give specific information on the infrastructure of the tumours of pancreas cancer.

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