





# Should pancreas cyst fluids be divided into two for cytological diagnosis and biochemical tests?

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## ABSTRACT

**Background/Aims:** The aim of the present study was to investigate whether pancreas cyst fluids should be divided into two for cytological diagnosis and biochemical tests.

**Materials and Methods:** The present study was conducted with fluids aspirated from 12 pancreas cysts. The fluids were divided into two and sent to the cytopathology (fluid 1) and biochemistry (fluid 2) laboratories. Fluid 1 was centrifuged at the cytopathology laboratory. Cytology slides were prepared from the deposit, and the supernatant was sent to the biochemistry laboratory. Fluid 2 was centrifuged at the biochemistry laboratory, and amylase, carcinoembryonic antigen, and cancer antigen 19.9 levels were determined in the supernatant. These procedures were repeated for fluid 1 from the cytopathology laboratory. The remaining fluid 2 was sent to the cytopathology laboratory. Fluid 1-like slides were prepared from fluid 2 in the cytopathology laboratory. Cytological diagnoses of fluid 1 and fluid 2 were compared, and the Pearson correlation coefficient for biochemical test results was identified.

**Results:** 92% of fluid 1 and 50% of fluid 2 were diagnostic. Biochemical test results of fluid 1 and fluid 2 were similar, and the Pearson correlation coefficient was high.

**Conclusion:** Our results showed that pancreatic cyst fluids did not need to be divided into two for cytological diagnosis and biochemical tests. Following centrifugation of the whole fluid at the cytopathology laboratory, the deposit and the supernatant can be used for cytological diagnosis and for biochemical tests, respectively. With this protocol, the sensitivity of cytological diagnoses and biochemical tests of pancreatic cyst fluids may increase.

**Keywords:** Biochemical tests, cytological diagnosis, pancreas cyst fluids

## INTRODUCTION

Pancreatic cystic lesions (PCLs) are increasingly diagnosed with the development and widespread use of high-resolution imaging techniques (1-3). PCL prevalence is reported to be 1%-15% in the general population (4-6). Pseudocysts and other non-neoplastic cysts were reported to be more frequent than neoplastic cysts in the past, however recent studies have revealed that most of incidentally recognized pancreatic cysts are neoplastic in nature (1,2,7,8).

The mucinous-non-mucinous differentiation is important in the diagnosis of neoplastic pancreatic cysts (3,4,8). Mucinous cystic lesions (MCLs) consist of mucinous cystic neoplasia (MCN) and intraductal papillary mucinous neoplasia (IPMN) (2-4,8,9). MCN and IPMN are premalignant neoplastic lesions, and surgical resection is used to treat lesions with high-grade dysplasia in the epithelium (4,9). Non-mucinous cystic lesions include pseudocysts, serous cystadenomas, cystic pancreatic endocrine neoplasms,

and solid pseudopapillary tumors (2,8,9). Diagnosis of the lesion and the benign-malignant and mucinous-non-mucinous differentiation are important for patient management in PCLs, whereas determining the degree of dysplasia of the epithelium is critical for MCLs (2,3,10). Endoscopic ultrasound-guided fine needle aspiration cytology (EUS-FNAC) is increasingly being used in the diagnosis of pancreas cysts to obtain such data (1-3,6-8,10).

The sensitivity of the EUS-FNAC diagnosis of PCL is low (1-3,11). The main reason is the hypocellularity of the cyst fluid (1,2,4). EUS diagnoses, hyperviscosity tests, and biochemical tests also have low sensitivity in the diagnosis of PCLs (1-3,11). However, the combined use of these tools is reported to increase both the sensitivity and accuracy (3-5,10,11). Molecular tests have been added to this combination in recent years, and DNA mutations in genes, such as K-RAS, GNAS, VHL, TP53, SMAD4, and CDKN2A, are investigated in the cyst fluid (2,4,5,10,11).

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Despite the increased sensitivity and accuracy of a PCL diagnosis with the current tools, the results are still not at the desired level.

Pancreas cyst fluids (PCFs) are currently routinely divided into two to send to the cytopathology and biochemistry laboratories in many centers (1,2,8). There are certain problems in this protocol as the cells in the fluid sent to the biochemistry laboratory cannot be used later for cytological diagnosis. Similarly, fluid sent to the cytopathology laboratory cannot be used for biochemical tests and is discarded. Dividing the fluid of small cysts is difficult, and endosonographers are forced to select between cytological diagnosis and biochemical analysis in some cases (1,2). Another problem is that biochemical tests cannot be studied in fluids with very low volume (1,3,8).

It has already previously been reported that cytological evaluation, biochemical tests, and molecular tests can be performed on the same PCF sample without dividing (12). However, there is no study comparing cytological diagnoses and biochemical test results of fluids sent to the cytopathology and biochemistry laboratories in the literature.

The aim of the present study was to find the answer to the following questions:

1. Should PCFs be divided into two for cytological diagnosis and biochemical tests?
2. Can the cells in the fluid remaining after biochemical tests be used in cytological diagnosis?
3. Can the volume of fluid remaining after cytology preparations be used for biochemical tests?

## **MATERIALS AND METHODS**

This prospective study was conducted with fluids aspirated under EUS guidance from the pancreas cyst of 12 patients during March 2017-June 2018. EUS-FNAC was performed in four different centers, and microscopic evaluation was performed in one center.

Deep sedation by an anesthetist was used for all patients during EUS-FNA. EUS-FNAs were performed by the Olympus UCT 140 linear scope (Olympus America Inc., Center Valley, PA, USA). A Cook Medical 22G needle was used for aspirations. The 19G needle was preferred for aspiration of large and dense (thick) cysts. To minimize the infection risk, an attempt was made to aspirate the entire cyst fluid at the same time. All patients were administered prophylactic antibiotics intravenously before

the aspiration. Oral antibiotics were continued for 3 days after the aspiration. After the EUS-FNA procedure was completed, the patients were kept under observation in the hospital for 2 h and then followed up at the outpatient department.

Aspirated fluids were divided into two with one part (fluid 1) sent to the cytopathology laboratory and the other part (fluid 2) sent to the biochemistry laboratory. Fluid 1 was concentrated by centrifugation at 1000 rpm for 3 min in the cytopathology laboratory. Four cytospin (Shandon 4 Cytospin) slides were prepared from the concentrated fluid, with one of them stained by May-Grunwald-Giemsa (MGG) and the others with Papanicolaou (PAP) stain. Two direct smear preparations were made from each case, and one of them was stained with MGG and the other with PAP. The particles within the deposit were fixed with 10% formalin, and a cell block was prepared. The cell blocks were cut into 5 µg thick and stained with hematoxylin and eosin. The supernatant remaining in the cytospin tubes was sent to the biochemistry laboratory. Cytology slides were also obtained from fluid 2 from the biochemistry laboratory at the cytopathology laboratory. Two senior cytopathologists (DS and IA) both provided the cytological diagnosis for the preparations from fluid 1 and fluid 2.

Fluid 2 was centrifuged at 2500 rpm for 5 min in the biochemistry laboratory, and amylase, carcinoembryonic antigen (CEA), and cancer antigen (CA) 19.9 levels were identified by using the supernatant. After the test results were received, the remaining fluid 2 was sent to the cytopathology laboratory. The same technical procedures conducted for fluid 2 in the biochemistry laboratory were also conducted for fluid 1 from the cytopathology laboratory. Similar to fluid 2, the amylase, CEA, and CA 19.9 levels in fluid 1 were identified.

The study was approved by the ethical committee at Acibadem University ATADEK (2018 2/7, approval date: 02/15/2018). Written informed consent was obtained from patient who participated in this study.

## **Statistical analysis**

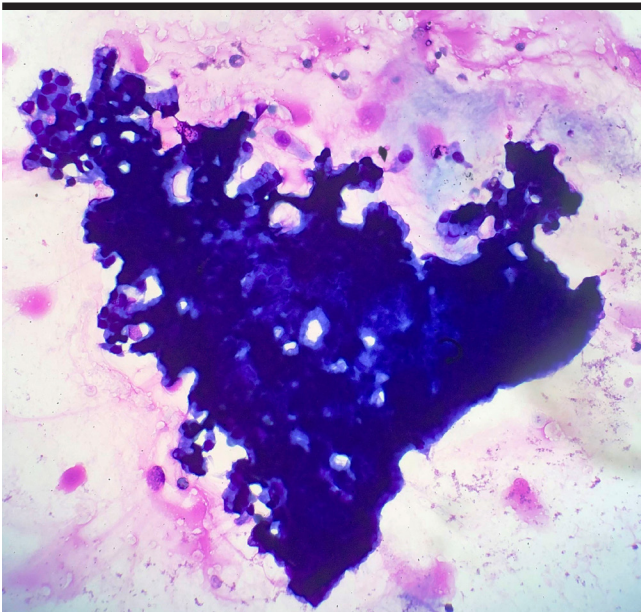
The data were analyzed using the Statistical Package for Social Sciences for Windows software version 21 (IBM Corp.; Armonk, NY, USA) (licensed by Istanbul University). Descriptive statistics were calculated for all outcomes variables (mean and percentage). The Pearson correlation coefficient was used to compare the amylase, CEA, and CA results of fluid 1 and fluid 2. A p-value of <0.05 was considered as statistically significant.

## RESULTS

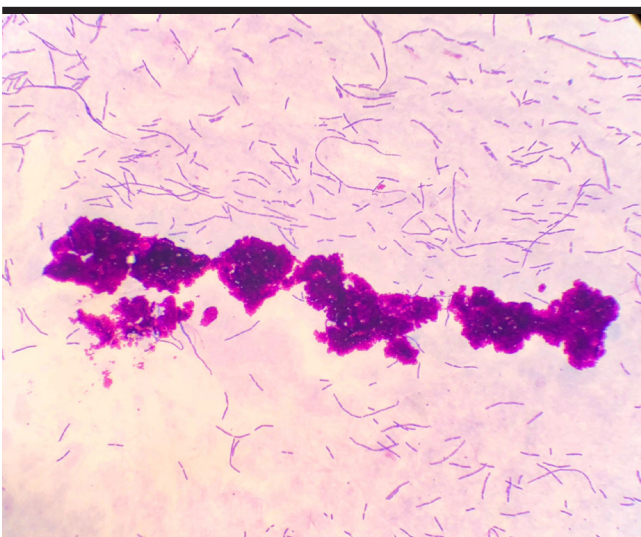
The age and gender of the patients, size and location of the cysts, volume of aspirated fluids, and number of aspirations are presented in Table 1.

While fluid 1 was more cellular than fluid 2 in 8/12 (67%) cases, cellularity was equal in 4/12 (33%) cases. The cy-

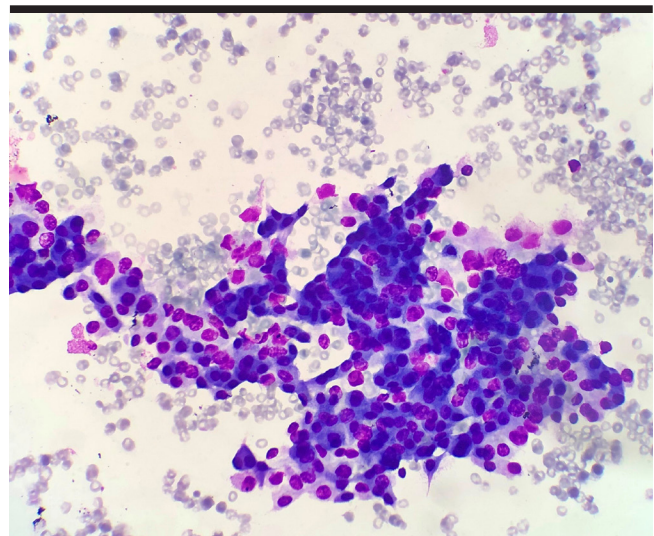
tological diagnoses of fluid 1 and fluid 2 were the same in 6/12 (50%) cases. While fluid 1 was diagnostic in 11/12 (92%) cases, it was 5/12 (42%) cases for fluid 2 (Table 2). Cytolysis and enzymatic digestion were present in the non-diagnostic cases (Figure 1-4). A large number of bacteria were seen in two and *Candida albicans* in one. The transportation durations of the fluids were 1-5 days for the cytopathology to biochemistry laboratory and 1-6 days for the biochemistry to cytopathology laboratory (Table 2).



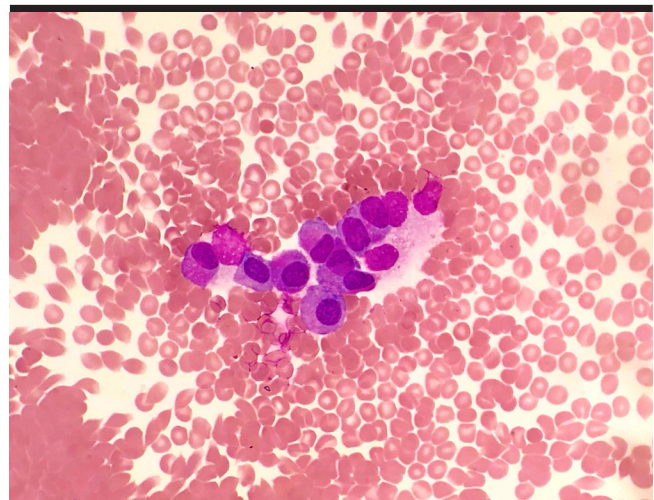
**Figure 1.** The slide was prepared from fluid 1. Papillary pattern of mucinous epithelium in IPMN (MGG, ×200 magnification)



**Figure 2.** Same case with Figure 1. The slide was prepared from fluid 2. Non-diagnostic cellular debris and bacterial contamination (MGG, ×400 magnification)



**Figure 3.** The slide was prepared from fluid 1. Uniform epithelial cell layers in a cystic pancreatic endocrine neoplasia (MGG, ×200 magnification)



**Figure 4.** Same case with Figure 3. The slide was prepared from fluid 2. A smaller epithelial cell group than Figure 3. Round nuclei and eccentric nuclear localization in cells (MGG, ×400 magnification)



**Table 1.** Clinical characteristics of the cases

Case	Age	Gender	Size of the cyst (mm)	Localization	Fluid volume (mL)	Aspiration no.
1	63	F	20	Head	2.0	1
2	40	F	42	Body	10.5	3
3	44	F	90	Tail	60.0	1
4	74	M	30	Head	3.0	2
5	68	F	40	Head	3.0	3
6	79	M	60	Body-tail	40.0	3
7	28	F	41	Body-tail	10.0	1
8	52	M	30	Head	7.0	1
9	59	F	25	Tail	2.0	1
10	40	M	55	Body-tail	10.0	1
11	64	F	30	Head	3.0	1
12	60	F	35	Tail	4	3

**Table 2.** Cellularity, cytological diagnoses, and transport durations of fluid 1 and fluid 2

Case	Cellularity	Cytological diagnoses		Transport time (day)	
		Fluid 1	Fluid 2	From C to B	From B to C
1	1>2	IPMN	ND	2	3
2	1=2	SC	SC	3	4
3	1=2	IPMN	ND	3	4
4	1=2	ND	ND	3	4
5	1>2	IPMN	IPMN	3	4
6	1=2	MCN	ND	4	5
7	1>2	Pseudocyst	ND	5	6
8	1>2	Pseudocyst	ND	2	3
9	1>2	SC	SC	2	3
10	1>2	Pseudocyst	Pseudocyst	3	4
11	1>2	IPMN	ND	1	1
12	1>2	CPEN	CPEN	1	1

C to B: cytopathology to biochemistry; B to C: biochemistry to cytopathology; IPMN: intraductal papillary mucinous neoplasm; MCN: mucinous cystic neoplasm; SC: serous cystadenoma; ND: non-diagnostic; CPEN: cystic pancreatic endocrine neoplasm

Biochemical test results of fluid 1 and fluid 2 were similar (Table 3). Pearson correlation coefficients were very high for the amylase, CEA, and CA 19.9 results of fluid 1 and fluid 2. P-values were slightly significant (Graphs 1-3). It was not possible to determine amylase in two cases and CEA in one case in fluid 1 due to technical reasons. The CA 19.9 level could not be determined in fluid 1 and fluid 2 in one case (Table 3). Table 3 presents the biochemical test results of fluid 1 and fluid 2. Pearson correlation coefficients were identified for these results (Graphic 1-3).

## DISCUSSION

EUS-FNAC is used increasingly more commonly in the neoplastic-non-neoplastic, mucinous-non-mucinous, and benign-malignant differentiation of pancreas cysts (1-3,6-8). PCF is currently divided into two for cytological diagnosis and biochemical tests in many centers (1,2,8). However, this procedure can be difficult, especially for small cysts. Biochemical tests could not be performed in 49% of the cases due to the lack of sufficient fluid in a study where the priority was cytopathological evaluation in the diagnosis (1).

**Table 3.** Biochemical test results of fluid 1 and fluid 2

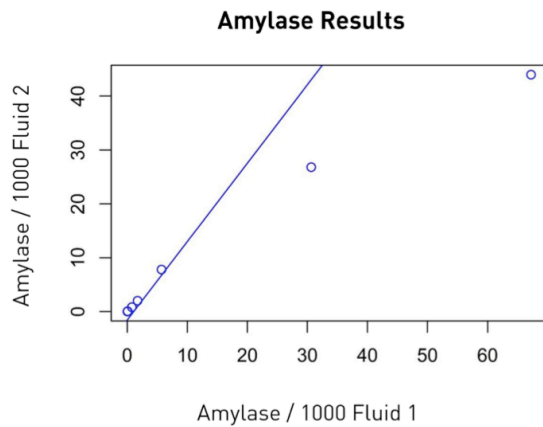
Case	Amylase (IU/L)		CEA (ng/mL)		CA 19.9 (IU/mL)	
	Fluid 1	Fluid 2	Fluid 1	Fluid 2	Fluid 1	Fluid 2
1	26,800	30,650	367	360	3336.45	340.00
2	21.8	14.3	0.17	<0.5	613.0	649.0
3	1717.5	2021.0	1714.0	1717.6	3,068,799.5	3,369,179.0
4	-	172.3	80.3	66.9	-	-
5	-	63,140.0	710.0	567.5	123,454.5	114,793.0
6	63.5	69.4	98,422.1	95,914.5	12,466,382.0	10,503,662.0
7	759.0	821.0	51.0	54.7	103,736.2	104,348.3
8	67,240.0	43,960.0	-	32.1	181.7	144.8
9	53.3	52.0	0.579	0.579	1.87	1.87
10	5700	7797	38.1	36.2	23,185.0	24,653.0
11	>2010	>2010	570	430	>40,000	>40,000
12	26.6	29.00	1.41	1.12	8.63	9.00

The diagnostic sensitivity of EUS, cytological diagnoses, and biochemical tests are low in pancreas cysts (1,2,11-13). The combined use of these tests has been reported to increase the sensitivity (3,4). Molecular tests were added to this combination in recent years (3-5,8,10). The main reason for the low sensitivity of cytological diagnosis for PCF is the hypocellular nature of the fluid (2,3,5,9). When the fluid is divided into two, the cells in the part sent to the biochemistry laboratory cannot be used for cytological diagnosis. Similarly, fluid sent to the cytopathology laboratory cannot be used for biochemical tests. PCFs are centrifuged similarly in the cytopathology and biochemistry laboratories, and the aim of centrifugation is to separate the fluid into two compartments to create a deposit containing the particles and cells at the bottom of the tube and a supernatant free of particles and cells in the upper part of the tube. Cytology preparations and a cell block are prepared from the deposit, whereas the supernatant is used for biochemical tests (12). The supernatant in the cytopathology laboratory and the deposit in the biochemistry laboratory are not used and are discarded.

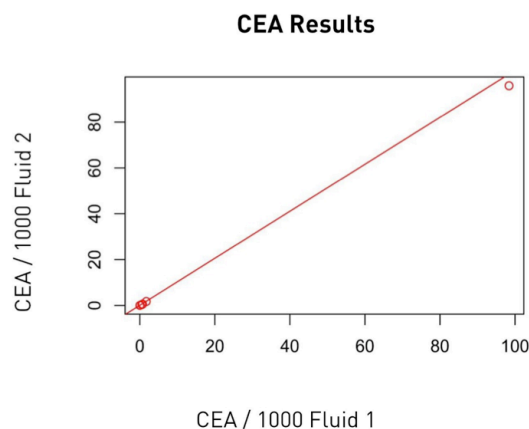
Fluid 1 was found to be more cellular than fluid 2 in 8/12 (67%) cases, whereas the cellularity was equal in 4/12 (33%) cases (Table 2). These results indicate that cells are present in fluid 2 sent for the biochemistry laboratory, but these cells currently cannot be used in cytological diagnosis. It would be possible to increase the sensitivity of cytological diagnosis by including these cells in the microscopic evaluation. Our results demonstrated that the compliance between the cytological diagnoses of fluid 1

and fluid 2 was low. The most important reason of this result was that fluid 2 was non-diagnostic in 7 (58%) cases (Table 2). Delays in the transportation of fluid 2 to the cytopathology laboratory after the biochemical tests were seen to cause cytolysis, enzymatic digestion, and bacterial and fungal contamination and therefore impair the diagnostic cytomorphological characteristics of the cells (Table 2 and Figure 1, 2). The non-diagnostic rate of fluid 2 may be decreased by conducting the biochemical tests right after the aspiration and preparing the cytology preparations without delay.

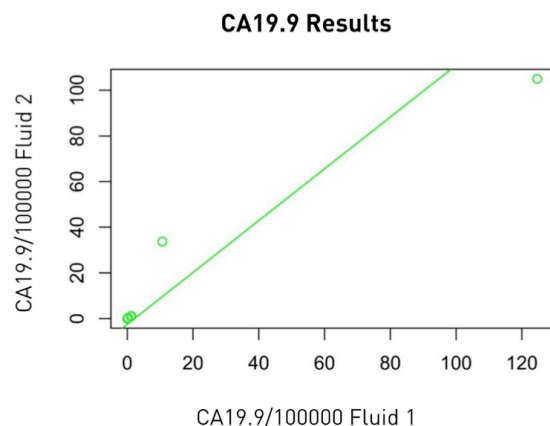
Biochemical test results of fluid 1 and fluid 2 were found to be rather similar, and the minor differences did not change the patient management as the Pearson correlation coefficients were high. P-values were slightly significant (Table 3 and Graphic 1-3). These results indicate that biochemical test results are not affected by the delays in transportation of fluid 1 from the cytopathology to biochemistry laboratory. The high degree of correlation between fluid 1 and fluid 2 biochemical test results shows that PCFs do not need to be divided for cytological diagnosis and biochemical tests. The entire fluid aspirated from the pancreas cyst should be sent first to the cytopathology laboratory. Cytology slides should be prepared from the deposit after centrifugation, and the remaining supernatant should be sent to the biochemistry laboratory for biochemical tests. The difficulty of dividing fluid from small cysts is eliminated with this protocol. Microscopic evaluation of all the cells in the entire fluid could also increase the sensitivity of the cytological diagnosis, whereas the increased volume of the supernatant could



**Graphic 1.** The Pearson correlation coefficient between fluid 1 and fluid 2 for the amylase test results was 0.99 ( $p=3.91 \times 10^{-6}$ )



**Graphic 2.** The Pearson correlation coefficient between fluid 1 and fluid 2 for the CEA test results was 0.999 ( $p<2.2 \times 10^{-16}$ )



**Graphic 3.** The Pearson correlation coefficient between fluid 1 and fluid 2 for the CA 19.9 test results was 0.97 ( $p=1.097 \times 10^{-5}$ )

increase the sensitivity of biochemical tests. Further studies should be conducted to prove the accuracy of these suggestions.

The low number of cases is the limitation of the present study. Considering similar centrifugation processes in the cytopathology and biochemistry laboratories, we believe that more study cases will support our results more strongly in the future.

In conclusion, PCFs do not need to be divided into two for cytological diagnosis and biochemical tests. The entire pancreatic cyst fluid should be sent first to the cytopathology laboratory, the deposit formed by centrifugation should be used for cytology slides, and the remaining supernatant should be sent to the biochemistry laboratory for biochemical tests. The current disposal of the supernatant at the cytopathology laboratory and the deposit at the biochemistry laboratory can be prevented with this protocol. The protocol can be routinely implemented with good cooperation between the endoscopy, cytopathology, and biochemistry laboratories. With this protocol, the sensitivity of cytological diagnoses and biochemical tests of pancreatic cyst fluids may increase.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Acibadem University ATADEK (2018 2/7, approval date: 02/15/2018).

**Informed Consent:** Written informed consent was obtained from patient who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept – D.Ş.; Design – D.Ş., B.Ç.; Supervision – D.Ş., İ.A.; Materials – D.Ş., B.Ç., G.Ş., N.T.; Data Collection and/or Processing – D.Ş., B.Ç., G.Ş., N.T.; Analysis and/or Interpretation – D.Ş., İ.A.; Literature Search – D.Ş.; Writing Manuscript – D.Ş.; Critical Review – D.Ş., B.Ç., İ.A., G.Ş., N.T.

**Conflict of Interest:** The authors have no conflicts of interest to declare.

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