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The perfect trio to decrease mortality in pancreatic cancers: Molecular marker screening tests with biosensors in precancerous lesions

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ABSTRACT

The mortality rates of pancreatic cancer (PC) are very high. The methods recommended in the studies conducted to date have been able to reduce mortality very little. This study aimed to review studies on this subject and to identify the most appropriate methods to decrease mortality in PCs. Therefore, research articles and reviews on this subject were analyzed intensely, and the findings were summarized. According to the results obtained in our study, to decrease mortality in pancreatic ductal adenocarcinomas, early diagnostic screening should be performed primarily with molecular markers and biosensors effective in precancerous lesions in risk groups. However, studies on such screening worldwide have not yet been conducted. Therefore, further research is needed to arrive at more precise conclusions.

Key words: Pancreatic cancer; Precancereous-precancerous-lesions; Molecular markers; Biosensors

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HIGHLIGHTS

To decrease mortality in PDACs;

- To minimize the prevalence of PDAC, it is first required to raise public awareness of non-genetic risk factors (such as excessive sugar consumption, smoking, obesity, and excessive alcohol use)
- Screening programs should be conducted using biosensors to detect noninvasive MMs that are effective in PCLs, especially in high-risk groups
- A definite diagnosis should be made with (EUS-FNAB) in suspicious cases during screening
- In cases of LGM PCL, the transformation of the tumor to HGM PCL should be prevented or slowed down, primarily with tumor suppressor MMs
- The cases with surgical indication should be operated in the early period.

INTRODUCTION

The mortality rate of pancreatic cancer (PC) is very high, and its incidence is increasing. In a study by Wu et al., according to data from the National Cancer Institute, while the incidence of PC was 11.85/100,000 in 2000, it increased to 14.70/100,000 in 2014, and incidence-based mortality increased from 9.96/100,000 in 2001 to 12.96/100,000 in 2014.[1] The lifetime PC incidence is approximately 1%.[2]

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According to 2017 data from the National Cancer Institute, 53,670 new PC cases and 43,090 deaths were observed in the USA. The 5-year survival rate can only increase to 8.2%.[3] However, in a study by Blackford et al., between 2004 and 2016, it was reported that 5-year survival in Stage IA cases increased to 85% in pancreatic ductal adenocarcinoma (PDAC) cases, and the diagnosis rates in these cases increased significantly from 11.4% to 17.7%.[4] Risk factors for PC include familial diseases, new-onset diabetes, diabetes mellitus, smoking, excessive alcohol consumption, obesity, advanced age, and chronic pancreatitis.[4]

In PDACs limited to the ductus epithelium and smaller than 1 cm, the 5-year survival rate was 100%. However, the tumor remained at this stage for only a few months.[5] According to the results of Kanno et al., in a meta-analysis, only 3% of PDAC cases were Stage 0 or Stage 1. The overall survival rate at 10 years was 93.7%. The 10-year survival rates were 94.7% in Stage 0, 93.8% in Stage 1A, and 78.9% in Stage 1B.[6]

The possible main reasons for the high mortality in PCs and the increasing incidence-related mortality are as follows:

1. Increasing number of non-genetic risk factors such as excessive sugar and alcohol consumption, smoking, and obesity in the society
2. The doubling time of cancer cells in PCs and the dwelling time between PC stages are very short, and diagnosis rates are low in the early stages
3. Limited imaging possibilities in the early stage due to the anatomical location of the pancreas
4. The reasons for the difficulty and high complication rates of operations performed on the pancreas are its anatomical localization, endocrine tissues, and fragile nature of the pancreatic tissue.

The most crucial mutant driver gene in PDACs is KRASG12D.[7] In a large-scale meta-analysis by Wang et al., KRAS mutations were reported to be very common in PDACs (94.9%) and gastrointestinal tumors.[8] KRAS mutations can be detected using non-invasive methods in patients with PDAC. In a study by Ako et al., cancer-specific KRAS mutations (G12D, G12V, and G12R) were analyzed in tissue, serum, and plasma samples of 40 PDAC patients. Cancer-specific DNA mutations have been detected in tissue samples in 93% of cases and in serum and plasma samples in 48%.[9]

In a study by Hsu et al., in mice, excess glucose and N-acetylglucosamine caused an increase in N-GlcNAcylation, causing genome instability and KRAS mutations, thus initiating PCs.[10]

Tien et al., explained why KRAS mutations are more common in PDACs as follows: They showed that phosphofructokinase activity in pancreatic cells is less than that in other cells, that in excessive sugar consumption, it increases O-Glc-Acylation and decreases ribonucleotide reductase activity, leading to dNTP deficiency, genomic DNA changes, KRAS mutations, and cellular transformation.[11]

According to the recommendations of the National Cancer Institute, to say that the screening methods used in the early diagnosis of PDAC are effective;

1. It should be able to be diagnosed early
2. It should be shown that it can reduce mortality
3. The benefits of the method used should outweigh the harms.[12]

There are many methods used in the early diagnosis of PDAC to date: Endoscopic ultrasonography-fine-needle aspiration biopsy (EUS-FNAB) is considered the gold standard in the early diagnosis of PDAC.[13] However, because it is an invasive method, it is challenging to use this method as a screening test.

In a study conducted in Japan, the correct diagnosis rates of ultrasonography, computed tomography, magnetic resonance imaging, and EUS in 200 Stage 0 and 1 early PDAC cases were found to be 67.5%, 98.0%, 86.5%, and 86.5%, respectively.[6] Contrast-enhanced EUS has been reported to be 62% effective in the differential diagnosis of pancreatic masses.[14] One of the biomarkers used for the diagnosis of PDAC for many years is CA-19. In a study by Jelski and Mroczko, CA-19 was still the most widely used biomarker for the early detection of PDAC. Analysis of specific glycans secreted by PC cells can be effective in diagnosis, but there is no single diagnostic method with high sensitivity and specificity, and it has been reported that combinations of many markers are needed.[15]

In a study conducted by Cohen et al., in 221 early-stage PDAC and 182 control group patients, the correct diagnosis rate was found to be 30% when the analysis of KRAS mutations in plasma cctDNAs was performed alone, while the rate of correct diagnosis was found with protein biomarkers (CA-19-9, CEA, and CA-125). When analyzed together, it has been reported to increase up to 64%.[16] According to the results obtained in a meta-analysis by Diab et al., on the roles of biomarkers (CA19-9 and CA125) still used in the diagnosis of PDAC in surgical decision-making, it was determined that these biomarkers analyzed in blood due to methodological heterogeneity were found to be ineffective in deciding the surgical method to be used.[17]

In a study by Ray, a 3-phase analysis in the serum of 20, 189, and 537 PDAC patients was performed with

thrombospondin-2 and CA-19-9. When they were used as a panel, a value of 0.96 for area under the ROC curve (AUC) was obtained, indicating their effectiveness in the early detection of PDAC. However, in this study, serum samples were obtained from all patients with PDAC, and no stage definition was made.[18] According to the findings obtained in the studies analyzed by Modi et al., it was reported that CA-19-9 reached high blood values even 2 years before the clinical PDAC disease. It has been reported that using other methods in longitudinal screening tests in the community is difficult and expensive; and therefore CA-19-9 is an ideal biomarker for screening.[19] However, when the articles reviewed by Modi et al., were examined, it was observed that most of the PDAC stages were not defined and there were no controlled studies.[19]

In a meta-analysis by Brezgyte et al., the sensitivity, specificity, and predictive value of CA-19-9 were reported to be very low (0.5–0.9%). In addition, when 4000 articles included in their studies were analyzed using the Quality Assessment of Diagnostic Accuracy Studies-2 tool, only 49 could be evaluated. According to the results of their research, newly developed noninvasive molecular markers (MMs) have been reported to be effective in the early diagnosis of PDAC. Still more studies are needed to include them in clinical practice.[20]

Bioinformatic tools and workflow studies were performed by Vandembrouck et al., to identify suitable biomarkers for early diagnosis in PDACs, and 24 candidate biomarkers were evaluated experimentally using mass spectroscopy -based proteomics. It was reported that three proteins (SYCN, REG1B, and PRSS2) were detected as effective biomarkers.[21] Nicoletti et al., in their review, said that early diagnosis of PDACs is challenging, extracellular vesicles, which are membrane-covered cellular products such as exosomes and microvesicles, are produced more in processes such as inflammation and tumorigenesis, and may have an essential role in the early diagnosis of PDAC.[22] In a study by Guler et al., changes in 5-hydroxymethylcytosine in circulating cell-free DNA in the plasma were analyzed in 64 PDAC and 243 non-PDAC patients. Accurate diagnosis could be made in PDAC cases with an AUC value of 0.92.[23] In a controlled study conducted by Zhou et al., on 20 PDAC patients, 1206 plasma lipid spectra were analyzed, and it was shown that nine lipids were significantly upregulated in PDAC patients and had the potential to be used in early diagnosis.[24] Hocker et al., showed that early-stage PDAC can be differentiated from chronic pancreatitis using the serum electrospray mass profiling method.[25]

Cao et al., analyzed 90 serum biomarkers in 28 Stage I PDAC cases and ten metabolite biomarkers in 53 tissue samples, and found that isoleucine and adrenic acid were upregulated at the same rate in serum and tissue samples with 0.93 AUC values.[26] Zawadzkaa et al., reported that inflammatory mediators such as C-reactive protein and matrix metalloproteinases may be effective biomarkers for the early diagnosis and prognosis of PDAC.[27]

In a study by Liu et al., the effects of the network of early diagnosis studies were investigated in the diagnosis of precancerous lesion (PCL) and early PDAC and were found to have a significant impact.[28] The methods to be used in the early diagnosis of PDAC and screening should be non-invasive because of their ease of application. In PDACs, highly accurate diagnosis rates can be obtained using the non-invasive liquid biopsy method, important information about the molecular structure of the tumor can be obtained, prognosis can be determined, and they can be used in advanced treatment.[29] MicroRNAs, circulating tumor cells, exosomes, and circulating tumor DNAs (ctDNAs) have been reported to be effective in liquid biopsies for early diagnosis of PDAC.[30]

Biodegradable, harmless nanoparticles can be used for the early diagnosis and treatment of PDAC and PCLs. In a study conducted by Smith et al., in mice, they developed a fluorescent polyplex nanoparticle and showed that since Cholecystokinin-B receptors are overexpressed in Pan1N lesions, this nanoparticle is retained by cholecystokinin receptors and localized only in Pan1N tissue, not in other organs.[31]

Despite efforts to increase early diagnosis rates and decrease mortality rates in PDAC, the rising incidence-related mortality rates indicate that new approaches should be introduced. For this purpose, the previous studies on this subject were reviewed, and a new roadmap that had not been defined before was created in our research.

WHY SHOULD SCREENINGS BE DONE FOR PCLS?

PDACs constitute 90% of PCs and 80% of PDACs develop from pancreatic intraepithelial lesions (Pan1Ns). Although other precursor lesions, intraductal papillary mucinous neoplasms (IPMN) and mucinous cystic neoplasms (MCN), can usually be detected by imaging techniques, cases of Pan1N are mainly detected incidentally on histopathological examinations.[32] In a study conducted in the USA, it was reported that 8% of people over 70 had PCL.[33] In another study by Gardner et al., PCL was found at a rate of 2.5% (approximately 6 million people) in the USA.[34]

Pan1N is a microscopic lesion and IPMN is a macroscopic lesion. Pan1N lesions are usually smaller than 1 cm in diameter, their incidence increases with age, and they account for 80% of invasive cancers. IPMNs are macroscopically

divided into three types: Central duct (MD), branch duct, and mixed duct (MT). IPMNs are histopathologically divided into four types: Gastric, intestinal, pancreaticobiliary, and oncocytic. Gastric types are low-grade malignancies (LGMs), intestinal types are intermediate-grade malignancies (IGMs), and others are high-grade malignancies (HGMs).[35] The transformation period of LGM IPMN and MCN (Pan1N1) to cancer was approximately 35 years, and 12 years of this period was the transition period to pan1N3. It takes approximately 6 years for IPMNs to become invasive. KRAS and Guanine Nucleotide binding protein (GNAS mutations) are common in IPMNs. In IPMN, 60% of GNAS and 80% of KRAS mutations occur.[36] Over time, mutations in tumor suppressor genes such as RNF43, CDKN2A, TP53, and SMAD4 transform lesions into high-grade IPMN and invasive cancer.[37,38] Cancers that develop from IPMN or are associated with IPMN (9–44%) have a better prognosis (mean survival; 21–58 months) than non-IPMN cancers (mean survival; 12–23 months).

Studies have shown a correlation between mutations in these genes, dysplasia, and subtypes. According to the results of genetic analysis studies, the transformation process of high-risk PCLs (new-onset diabetes, obstructive jaundice, 5 mm > mural nodule, central pancreatic duct >10 mm, pancreatitis, 3 cm > cyst size, thick cyst wall, sudden enlargement of the pancreatic duct) to cancer is approximately 3 years.[39]

Chen et al., reported that PCLs have different clinicopathological, endomicroscopic, and molecular features, and risk classification can be made with EUS-FNA.[40] Keane et al., obtained a sensitivity of 90% in IPMNs and 100% in PDAC with the needle-based confocal laser endomicroscopy (EUS-nCLE) technique.[41] In a study by Napoleon et al., the sensitivity and specificity were >95% in nCLE and 206 PCLs.[42] It has been reported that with targeted metabolomic and lipidomic tests, HGD MCNs can be differentiated from serous cysts with 100% accuracy and invasive cancers with 90% accuracy.[43]

In a study by Chidambaram et al., KRAS, GNAS, VHL, PIK3CA, SMAD4, and TP53 gene mutation analyses were performed in cyst fluid in PCLs, and ctDNAs were detected by noninvasive methods in Pan1N cases.[44] In PDACs, the transition period from normal cells to PCL and from PCL to PDAC is quite long, whereas the doubling time of cancer cells and the dwelling time from Stage 0 to Stage 4 are very short. Therefore, it is difficult to diagnose early and is an important cause of high mortality. The transition order from normal cells to cancer cells in most cases of PDAC is Pan1N1 > Pan1N2 > Pan1N3 > PDAC.

According to the results of a simulation model made by Peters et al., on the transition to PDACs, the transition period from Pan1N1 to PDAC was approximately 35 years, and from Pan1N3 to PDAC approximately 11 years.[2] According to the results of a study conducted by Yu et al., by analyzing the age, tumor size, stage, and demographic information of 13,131 PDAC patients in the USA; it has been calculated that it starts at an earlier age in African-American male people, the transition time from T1 to T4 is 1.3 years, from T1 to T2; 0.79 years, the transition period from T1 to T3 is 1.06 years, from T3 to T4 0.24 years.[45] In 900 PDAC cases, the mean survival in the earliest stages (T1N0) was 38 months and the mean survival in the latest stages was 11 months.[46]

At the time of diagnosis of PDAC, 90% of the tumors spread beyond the pancreas, 50% have metastases, and 15–20% are resectable.[47] In a study conducted by Salvia et al., main duct Stage I PDAC was detected in 58 of 140 IPMN cases, and 10-year disease-free survival was found in 100% of patients without PDAC and 60% of patients with PDAC.[48] Dwelling time from Stage 0 (undiagnosed PDAC) to Stage 1 (diagnosed PDAC); for approximately 2.5 years. This long process is advantageous for early diagnosis. Although there are experimental studies on the measurement of tumor growth rates in different cancer types, it is possible that the results obtained in these studies are not valid in humans. Therefore, to determine the growth rate of the tumor in PDACs; Yu et al., developed a model that the time required for PDAC to go through different stages may be related to the ages of patients diagnosed at different locations. For this purpose, data from the National Cancer Institute between 2004 and 2011 were analyzed. According to the results obtained, the growth rate of the tumor is very high in PDACs, starting from the stages of localized or localized advanced cancers, and the average transition time from T1 to T4 is 14 months.[45] In a mathematical model developed by Sun et al., by considering DNA mutations, it was shown that tumor doubling time in colorectal cancers is 1 year in Mx34 cases, 1.5 years in Co82 cases, and 5–11 years are required for the transformation from large adenoma to cancer.[49]

In a study by Dahan et al., the doubling time was 103 days in triple-negative breast cancers (BC) and 241 days in HER2+ BCs.[50] As seen in these studies, the doubling time of cancer cells and dwelling time in PDACs are much shorter than in other common cancer types.

Malignant transformation in PCLs can be slowed by interfering with signaling pathways involving tumor suppressor MMs. In a study by Li et al., it was reported that by manipulating signaling pathways such as the MAPK, Wnt, Notch, and PI3K/Akt pathways, PanIN and even PDAC can be reprogrammed into normal cells (clinical chemoprevention).[51]

Table 1: MMs for non-invasive diagnosis in IPMNs

Reference	No. of Cases	MM	Non-invasive method	Pathology	Efficiency
Goto et al.[70]	29	Exosomal miRNA-191	qRT-PCR in serum	IPMN	Sensitivity: 0.64 Specivity: 0.79 AUC: 0.741
		Exosomal miRNA-21	qRT-PCR in serum	IPMN	Sensitivity: 0.75 Specivity: 0.81 AUC: 0.741
		Exosomal miRNA-451a	qRT-PCR in serum	IPMN	Sensitivity: 0.62 Specivity: 0.85 AUC: 0.742
Li et al.[64]	20	miRNA-1290	qRT-PCR in plasma	IPMN	AUC: 0.760
Peruth-Way et al.[71]	42	30 miRNA-signature	qRT-PCR in plasma	IPMN	AUC: 0.744
Hata et al.[72]	34	GNAS mutations in cell-free DNA	dd-PCR in serum	IPMN	Positive rate: 32.3
Slater et al.[73]	5	miRNA-196a, miRNA196b	RT-PCR in serum	Pan 1N 2/3	AUC: 0.99
Akimoto et al.[74]	79	N-glycan profiles	Immunoradiometric assay in serum	IPMN	AUC: 0.803
Watanabe et al.[75]	17	Dermokin	RT-PCR in serum	IPMN	Positive rate: 76.4
Sakai et al.[76]	23	mRNA screening	RT-PCR in blood	IPMN	Positive rate: 52.1
Abue et al.[77]	12	miRNA-21	TaqMan micro RNA kit in plasma	IPMN	AUC: 0.73
Berger et al.[78]	21	GNAS mutations in cell-free DNA	dd-PCR in serum	IPMN	Sensitivity: 100% Specivity: 26

IPMN: Intraductal papillary mucinous neoplasm, qRT-PCR: Quantitative real-time polymerase chain reaction, dd-PCR: Droplet digital polymerase chain reaction, RT-PCR: Reverse Transcription polymerase chain reaction, TaqMan Micro RNA RT Kit: TaqMan Micro RNA reverse transcription kit, GNAS mutation: Guanine Nucleotide binding protein, Alpha Stimulating gene mutation, MMs: Molecular markers, AUC: Area under the ROC curve

To reduce mortality in PDACs, it will be advantageous to perform screening and management of PCLs. Because;

1. In PDAC, the dwelling and doubling time of cancer cells are very short compared to other cancer types. For this reason, it is unlikely that an accurate diagnosis will be made in screening tests performed once a year for early-stage PDACs. Because PCLs take a long time to transition to PDAC, they are much more likely to be diagnosed without progression to PDAC, even if the correct diagnosis rates are lower in screening tests than in PDACs (Table 1). For example, if the sensitivity of an MM is 40% in HGM PCLs within 3 years, which is approximately the conversion process to PDAC, according to the binomial probability distribution formula:

$$P(x) = \left(\frac{n}{x}\right) p^{x} (1-p)^{n-x}$$

(Here n is the number of trials, p is the sensitivity of the marker, and x is the number of success expectations to be achieved in each test).

Probability of being diagnosed at 1 year; 40%,

Probability of diagnosis at 2 years: 64%,

The probability of being diagnosed at 3 years will increase to 78.4%.[52]

In a recent study, it was shown that early diagnosis rates can be increased and mortality can be reduced by miRNA-21 analysis in feces or plasma, since the early diagnosis process is longer, although PCLs have lower sensitivity rates in screening tests to be performed in colorectal cancers.[53]

2. Disease-free survival is long after operations performed on PCLs. In PDACs, the average survival is not very high even in T1N0 cases.
3. With non-surgical treatment methods in PCLs, the transformation into cancer can be slowed down and reversed.

There are three significant advantages of non-surgical treatment methods in PCLs:

i Perioperative morbidity (20–40%) and mortality (1–3%) are high even in operations performed in PCLs due to the localization of the pancreas, fragility of pancreatic tissue, and presence of endocrine tissues in the pancreatic tissue.[54,55]

ii The proportion of PCL in the community is very high. According to the recommendations of the American Gastroenterology Association, if all MCNs and IPMNs have solid components or if the canal is >5 mm, an operation is required.[56] Surgical treatment in these cases is difficult and costly.

iii Non-surgical methods have little effect on prolonging survival in PDACs, even in the early stages.

WHY SHOULD NON-INVASIVE MMS BE USED FOR EARLY DIAGNOSIS?

As in many cancer types, articles report that high sensitivity and specificity values are obtained with non-invasive MMs

in screening tests for early diagnosis of PDACs. In a study by Acar and Ozer, non-invasive serum MMs were more effective than invasive methods for the early diagnosis of BCs.[57] In a study by Iovanna, an essential reason for the high mortality in PDAC was the lack of early diagnosis, as well as the molecular heterogeneity of the tumor.

Therefore, it has been reported that early diagnosis can be made using effective non-invasive diagnostic methods such as cell-free DNA, circulating RNA, and methylated DNA in serum, and individual treatment can be performed by determining the molecular structure of the tumor.[58]

In a study by Kunovsky et al., it was reported that non-invasive miRNAs are the most effective and cost-effective method for early diagnosis of PDAC.[59] Tarasiuk et al., said that miRNAs play an essential role in the epithelial-mesenchymal transition (EMT) process, that metastases cannot develop before this process is completed in PDAC metastasis, and that the miR-21, miR-10, and miR-200 families play an essential role in this EMT process. In addition, tests with miRNAs are the most effective method for the early diagnosis of PDAC, and which it has been reported that using noninvasive, upregulated MMs as a panel will be more effective. The miRNAs significantly upregulated in the early diagnosis of PDAC are Ex-miR-21, Ex-miR-155, miR-182–5p, and miR-4732-5p.[60] Song et al., reported that early diagnosis of PDAC can be made, as in many cancer types, by analyzing MMs such as cfDNA, miRNA, and intercellular exosomes in human saliva as a non-invasive method.[61] Zhou et al., reported that the ideal MMs for early diagnosis of PDAC are mRNAs, especially lncRNAs, because they are stable and easy to detect.[63]

In a study by Fathizadeh et al., as in many cancer types, circRNAs (CircZMYM2, Hsa-circ-100782, Circ-0007534, Hsa-circ-0001649, Circ-IARS, CIRS-7, Circ-RHOT1, Circ-PDE8A, Circ-LDLRAD3, Circ-0030235, Hsa-circ-0006215, and Hsa-circ-0000977) affected the signaling pathway of tumor suppressor miRNAs in PDAC and influenced the cell cycle, tumorigenesis, and apoptosis. Due to these effects, it has been reported that they can be used in tumor suppression and prognosis.[63]

Li et al., performed miRNA analysis with the quantitative real-time polymerase chain reaction (PCR) TaqMan Micro RNA method in the serum of 735 PC and control cases; miR-1290 showed the best performance with 0.96 AUC values (sensitivity:0.88, specificity: 0.84). They also reported that the AUC values of miR-24, miR-134, miR-146a, miR-378, miR-484, miR-628-3p, and miR-1825 were more significant than 0.70 compared to healthy controls.[64] In this study, serum miRNA-1290 expression was significantly higher in IPMN cases than in the control group (AUC:0.76). AUC was found to be higher in PDAC cases than in IPMN cases and higher in IGM and HGM IPMNs (AUC: 0.82) than in LGM IPMNs.[64]

According to a study by Prinz et al., dysregulation of panel miRNAs (miR-31-5p, miR-483-5p, miR-99a-5p, miR-375) is effective in the diagnosis of PCLs, compared to those effective in solid PDAC tumors (miR-146, miR-196a/b, miR-198, miR-217, miR-409, and miR-490), which have been reported to have completely different expression.[65] In a study by Satoh, blood miR-1290 analysis was said to be the most effective miRNA for the early diagnosis of PDAC.[66] Zhang et al., showed that 30 early-stage PDAC, 30 chronic pancreatitis, and 30 healthy control cases could be diagnosed correctly with a 4-panel miRNA (MBD3L2, KRAS, ACRV1, and DPM1) in saliva with 0.97 AUC, 90% sensitivity, and 95% specificity.[67] In a study by Ganepola et al., it was shown that early diagnosis can be made with a three blood-based panel, miR-885-5, miR-22-3p, m.R-64-2b, with 91% sensitivity and specificity and 0.97AUC values in early stage PDACs.[68] In a study by Shao et al., the overexpression of microRNA-483-3p in the PDAC and Pan1N PCLs serum was compared to that in normal control subjects.[69] The MMs for the non-invasive diagnosis of IPMNs are shown in (Table 1).

As shown in (Table 1), the number of articles in which diagnostic tests performed with MMs in IPMN using non-invasive methods have been published is very few. In addition, the number of cases used in these studies and the efficacy rates achieved are generally low.

Genetic and transcriptomic MMs can be diagnosed with high AUC values (0.87) in PDACs.[33,37] In a study, analysis of 14 methylated DNA markers (NDRG4, BMP3, TBX15, C13orf18, PRKCB, CLEC11A, CD1D, ELMO1, 1GF2BP1, YRY2, ADCY1, FER1L4, MX1, and LRRC4) in pancreatic fluid was performed using a quantitative allele-specific real-time target and signal amplification method in 73 control groups consisting of 38 PDAC and high-grade IPMN cases and 41 low-grade 32 standard pancreatic cases.[79] With C1orf18, FER1L4, and BMP3 triple panel analysis, it has been shown that a correct diagnosis can be made with a sensitivity of 80% in 38 HGM and early-stage PDAC cases and 83% in advanced-stage PDAC. In addition, a study conducted with covariate analysis showed that the obtained results did not affect the correct diagnosis rates by age, sex, and tumor localization.[79]

This study made a 100% correct diagnosis in three IPMN and HGM cases with a triple panel. MMs have advantages in the early diagnosis of PDAC or PCL, and inhibiting the progression and proliferation of neoplastic cells: LncRNA GASS down-regulates miR-181c-5p and inhibits cancer

cell cycle, progression, and proliferation.[80] LINCOO673 lncRNA disrupts cell homeostasis by downregulating miR-504 and miR-23 and inhibiting the cell cycle, progression, and expansion.[81] LINCO1111 lncRNA inhibits cell cycle, proliferation, progression, and migration, and stops tumorigenesis by regulating DOSP1 expression through miR-3924.[82] IPMNs and MCNs constitute 8% of PDACs. Analysis of GRAS, KNAS, VHL, PIK3CA, SMAD4, and TP53 MMs in cyst fluid and needle-based confocal laser endomicroscopy have significantly increased the rate of accurate diagnosis.[35] In a study by Shen et al., to differentiate early PDAC cases from other cancer types, they analyzed the serum of 24 PDAC patients and 24 healthy controls with cell-free methylated DNA immune precipitation and high-throughput sequencing technique, and they were able to make an accurate diagnosis in early PDAC patients with 0.90 AUC values.[83] In a study by Hata et al., 34 out of 57 histologically diagnosed PCLs were identified as IPMN. In these 34 IPMN cases, GNAS and KRAS mutations in cDNAs were investigated using next-generation sequencing; GNAS mutations were found in 11 (32%) cases, and KRAS mutations were found in 2 cases (6%).[72]

According to the findings of these studies, MMs are used in the early diagnosis of PDACs.

1. It will significantly increase the sensitivity and specificity (Table 1)
2. By determining the molecular structure of the tumor, it will be determined whether it is a heterogeneous structure, which will contribute to the prediction of prognosis and chemotherapy
3. By disrupting the homeostasis of tumor cells, the advantage of inhibiting cell cycle, progression and proliferation will be obtained.

WHY SHOULD BIOSENSORS BE USED IN EARLY DIAGNOSIS?

Although PCR and enzyme-linked immunosorbent analysis are generally used for the early diagnosis of PDAC and PCL in the study of MMs, these tests are expensive, time-consuming, have low sensitivity and specificity, require expert personnel, and are not real-time tests.[62]

In recent years, biosensors have been used very effectively for the early diagnosis of cancer in many sectors. A review reported that biosensors are more effective, cost-effective, real-time, and easy for the early diagnosis of cancer than other methods.[86] Biosensors are a cheap, real-time method for the early diagnosis of cancer, which can be made in samples taken from body fluids by non-invasive methods and can detect electrochemical, colorimetric, and optical signals in biological structures such as DNA and RNA, with very high detection limits, very fast, with high sensitivity, specificity, and selectivity.[84-87] The sensitivity of biosensors in detecting MMs is so high that even a few million tumor cells can be seen with biosensors; however, other methods can detect at least one billion tumor cells.

Sharifianjazi et al., developed nanosensors that can detect nanomaterial-based markers in many cancer types.[88] Dorosty et al., reported that MMs such as miR-18a, miR-21, miR-196a, miR-1290, miR-492, and miR-196b were detected in PDACs using electrochemical-based biosensors in the early period.[89] In a study by Wang et al., it was reported that detecting hyaluronidase with multicolor biosensors in the serum of PDAC cases can be diagnosed within 40 min with the color change that occurs.[90] A study by Qing et al., reported that markers can be detected with high sensitivity, specificity, and selectivity using photoelectrochemical biosensors in early cancer diagnosis.[91]

With biosensors in PDAC, the sub-picomolar detection limit of mRNA-196b was decreased to 105 ± 4.1% in absolute human serum by the dual amplification method.[92] Using DNA tetrahedral nanostructure-based electrochemical miRNA biosensors, influential panel of mRNAs (miR-21, miR-155, miR-196a, and miR-210) can be investigated simultaneously in diagnosing PDAC.[93]

In a study by Qian et al., they reported that the presence of effective biomarkers in Pan1N and early PDACs, the detection of biomarkers with biosensors, and data analysis can enable early diagnosis in three stages, and the most effective biosensors are electrochemical and optical biosensors.[94] In a study by Chen et al., KRAS-12D and 13V mutations were detected with 86.2% sensitivity and 96.9% specificity due to DNA analyses performed with nanoprobe-based biosensors in plasma and fecal samples of 58 PDAC cases.[95]

Although there are some studies in which MMs are detected with high sensitivity and specificity using non-invasive methods with biosensors for the early diagnosis of PDAC, no analysis has been found on PCLs.

As can be seen in the results obtained in studies using biosensors in the early diagnosis of cancer, the detection of MMs with biosensors in the early diagnosis of PDAC and PCL has the following advantages:

1. Higher sensitivity, specificity, and selectivity can be obtained compared to other methods
2. Real-time results can be obtained with biosensors
3. It is a cost-effective method
4. It does not require expert personnel in its use
5. They are easy to transport and can be used easily in screening.

As seen in the articles analyzed in our study, mortality can be decreased by early diagnosis when effective non-invasive MMs in the PCL stage are detected with biosensors.

CONCLUSION

According to the results obtained in our study, to decrease mortality in PDACs, early diagnostic screening should be performed primarily with MMs and biosensors effective in PCLs in risk groups. However, studies on such screening worldwide have not yet been conducted. Further research is needed to arrive at more precise conclusions on this subject.

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