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The Diagnostic Value of Serum Glypican-3 in the Diagnosis of Hepatocellular Carcinoma: A Systematic Review and Meta-Analysis

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ABSTRACT: Background and aim: Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related deaths worldwide. It is curable in the early stages but has a poor prognosis when advanced. Current diagnostic protocols for the early stages of HCC are inefficient. Serum Glypican-3 (GPC-3) is a promising serum tumour marker in diagnosing HCC, and its diagnostic value has been explored in various studies. The outcomes, however, continue to be inconsistent and controversial. This systematic review and meta-analysis aimed to investigate the diagnostic accuracy of serum GPC-3 in hepatocellular carcinoma. This study included recent papers not captured in previous meta-analyses.

Method: PUBMED, MEDLINE, Cochrane Library, and CINAHL databases were systematically searched according to preferred reporting items for a systematic review and meta-analysis of diagnostic test accuracy studies guidelines. Studies on the diagnostic accuracy of serum GPC-3 in HCC were selected. The Quality assessment of diagnostic accuracy studies 2 (QUADAS-2) tools were used to assess the quality of selected studies. Sensitivity, specificity, and other diagnostic parameters were pooled using the random effect model. The Summary receiver operating characteristic (sROC) was used to summarise the diagnostic accuracy of Serum GPC-3.

Results: Eleven studies were included in this systematic review. The pooled sensitivity, specificity, and 95% confidence intervals (CIs) were 0.60 (0.57-0.62) and 0.67(0.64-0.70), respectively. The pooled positive and negative likelihood ratios and 95% CIs were 2.11 (1.51-2.93) and 0.59 (0.51-0.68), respectively; the pooled diagnostic odds ratio and the 95% CI were 4.07 (2.37-6.98), the area under the sROC curve was 0.7194.

Conclusion: This meta-analysis has shown that Serum GPC-3 has an acceptable diagnostic value in diagnosing HCC compared to benign chronic liver diseases. A higher-quality study with a larger sample size is necessary to thoroughly assess the diagnostic utility of serum GPC-3.

KEYWORDS: Glypican 3, Chronic liver diseases, Diagnostic accuracy, HCC, Biomarker.

INTRODUCTION

Liver cancer is the seventh most common cause of cancer and the second leading cause of cancer-related death worldwide in 2020 [1]. The most prevalent kind of liver cancer is hepatocellular carcinoma (HCC), accounting for around 90% of cases [2]. The prognosis of HCC varies significantly depending on the tumour stage at the time of diagnosis; as a result, early identification is essential to enable successful therapeutic intervention and hence increase patient survival [3]. When diagnosed early, HCC can be treated successfully by surgical resection [4]. Thus, there is a need for a diagnostic protocol that will ensure early detection of HCC to enable early treatment and a better outcome for patients.

The European Research Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) recommended liver imaging such as computed tomography (CT) and magnetic resonance imaging (MRI) for the diagnosis of HCC. British National Institute for Health and Care Excellence (NICE), in addition to MRI and CT, also included contrast-enhanced ultrasound (CEUS) in their recommendation for the diagnosis of HCC [5]. But, imaging examination, an outstanding accurate indirect diagnostic tool, relies on the radiologists' and doctors' abilities and expertise and is too expensive for widespread use for HCC screening and diagnosis [3, 6-8]. Secondly, imaging modalities pose limitations in diagnosing small HCC nodules; in that the sensitivity of CEUS, CT, and MRI to HCC nodules smaller than 2 centimetres is limited [9]. According to Hennedige & Venkatesh, (2013), using CEUS, CT, or MRI alone can only detect the typical features of HCC in about 26–62% of HCC nodules that is 1–2 cm in size [6]. Therefore, imaging modalities like CT, MRI, and CEUS are not efficient in diagnosing HCC at the early stage (detecting nodules less than 2 cm in size).

Biopsy and histopathological testing currently have a limited role in diagnosing HCC and have largely been replaced by CT, MRI, and CEUS. Biopsy and histopathology are primarily used to confirm HCC, especially in equivocal cases. However, EASL recommends histological diagnosis for nodules 1-2 cm in size with atypical features. Biopsy in HCC is fraught with many shortcomings, including; needle track seeding of malignant cells, sampling error, technical problems, bleedings and pains [6, 10].

Blood biomarkers measurements are also a reliable approach to making diagnoses and facilitating the screening of HCC. Their accessibility, non-invasiveness and lower cost make them ideal for cancer screening [8]. Alpha-Fetoprotein (AFP) is regarded as the most frequently utilised HCC biomarker globally for serological testing of HCC. However, its use has proved contentious; AFP analysis is unable to identify around 30% to 40% of patients with HCC, which results in treatment delays. AFP is elevated in people with benign liver conditions and has a poor positive predictive value (PPV) of 25.1% [7, 8, 11]. Therefore, a new biomarker with high diagnostic accuracy that might replace AFP or enhance AFP's diagnostic performance would be beneficial [12].

Glypican-3 (GPC-3) is a heparan sulphate proteoglycan that is connected to the cell membrane via glycosylated phosphatidylinositol [13]. The GPC-3 gene is found on chromosome X (Xq26.2). GPC-3 is thought to be a key regulator of cellular proliferation in embryonic mesodermal organs through the use of the Wnt, hedgehog, bone morphogenic protein, and fibroblast growth factor signalling pathways. It controls the rate of cell proliferation and cell death in some cell types during development [14]. GPC-3 is abundantly expressed throughout the placenta and the embryo's liver, lungs, and kidneys. In healthy adults, however, it is barely detectable in most organs. GPC-3 expression was reported in many adult cancers, including HCC, Lung cancer, melanomas, gastric cancer and also in Paediatric embryonal tumours. Among these cancers, it was found to be exceptionally high in HCC. Evidence indicates that GPC-3 participates in Wnt/β-catenin signalling and promotes cell proliferation in HCC cells. [13, 14].

Numerous studies have examined the role of GPC-3 in tumours, including its role in HCC. GPC-3 expression in HCC has been targeted for the treatment of HCC (utilising a recombinant humanised anti-GPC-3 monoclonal antibody) [14]. HCC vaccine therapy using GPC-3-derived peptide vaccine is also attracting serious research interest [14-16]. In a case-control study, Taniguchi et al. (2020) showed that in patients whose immunohistochemical staining for GPC-3 is positive; postoperative adjuvant vaccination with "GPC-3 peptide vaccine" may lower the 1-year recurrence rate and lengthen the overall survival. They also reported that GPC-3 peptide vaccination produces certain cytotoxic T lymphocytes and most probably can benefit patients [17]. A preclinical trial using GPC-3-coupled lymphocytes for treating HCC has also been performed with a positive result [18].

GPC-3 has also been found useful in the diagnosis of HCC. The high expression of GPC-3 in HCC cells has attracted a large number of studies on the usability of GPC-3 for the diagnosis of HCC [19]. In a study, Wang et al. (2006) demonstrated that GPC-3 can serve as a trustworthy immunohistochemistry marker for the detection of HCC [20]. GPC-3 messenger RNA has also been investigated and has shown a promising result as a reliable marker for diagnosing HCC [21].

GPC-3 is released into the bloodstream, making the serum level of GPC-3 a key indicator for the non-invasive detection of HCC [19]. Surprisingly, growing evidence indicates that some HCC patients who test negative for AFP (serum AFP < 400μg/L) are positive for GPC-3 [22]. Li et al. (2013) found that 48.8% of patients who test negative for serum AFP are positive for serum GPC-3 [22]; this may indicate GPC-3's promising potential and benefits over AFP in the diagnosis of HCC. It is noteworthy that there is no correlation between GPC-3 levels and AFP [23], suggesting that GPC-3 may serve as an independent biomarker from AFP.

Due to increasing evidence supporting the use of serum GPC-3 as a non-invasive marker for the detection of HCC, several researchers have carefully explored this topic in connection to the diagnostic potency of serum GPC-3, but their results, though promising, have been inconsistent and controversial [24-28]. In this systematic review and meta-analysis, the objective is to compile and evaluate the findings from investigations that assessed the diagnostic efficacy of serum GPC-3 in the diagnosis of HCC. Many systematic reviews and meta-analyses have already explored the diagnostic value of serum GPC-3, but with still conflicting and controversial results [7, 29-34]. The novelty of this current meta-analysis includes: Firstly, this meta-analysis included only studies that used individuals with (non-malignant) chronic liver diseases [35] in the control group, unlike some of the previous meta-analyses on this topic [7, 33]. The merit of using individuals with chronic liver disease as control is that HCC rarely occurs in people without chronic liver disease (healthy individuals) [3]; hence, healthy individuals may not be the right control group. Secondly, the most recent meta-analysis on the diagnostic value of serum GPC-3 in HCC was published in 2019 [29]. And since then, new studies have been published on the diagnostic value of serum GPC-3 [24-27, 36]. These new studies were captured in this meta-analysis. Thus, in addition to exploring this controversial issue, this meta-analysis will include the most recent studies on the diagnostic value of serum GPC-3 in HCC.

MATERIALS AND METHODS

This systematic review and meta-analysis is reported in accordance with the preferred reporting items for a systematic review and meta-analysis of diagnostic test accuracy studies (The PRISMA-DTA) Statement [37]

SEARCH STRATEGY

A comprehensive search of PUBMED, Cochrane Library, CINAHL and MEDLINE databases was performed to look for eligible studies. All eligible studies published from the inception of the respective databases till the date of search (16th July 2022 for MEDLINE and 26th June 2022 for the other databases) were selected. The following keywords and synonyms were used to find available articles: Value or benefits or importance, and diagnosis, or diagnosing or diagnostic or screening and Glypican-3 or "glypican 3" or glypican and "hepatocellular carcinoma" or "hepatocellular cancer" or "liver cancer" or "hepatic cancer" or "liver tumour". The search strings as copied out from the databases are shown in table 1. No filters were applied.

Table 1. Showing search strings used for literature search as copied out from databases

Databases searched	Search strings
PUBMED	(((Value[Title/Abstract] OR benefits[Title/Abstract] OR importance[Title/Abstract]) AND (Diagnosis[Title/Abstract] OR diagnosing[Title/Abstract] OR diagnostic[Title/Abstract] OR screening[Title/Abstract])) AND (Glypican-3[Title/Abstract] OR "glypican 3"[Title/Abstract] OR glypican[Title/Abstract])) AND ("Hepatocellular carcinoma"[Title/Abstract] OR "hepatocellular cancer"[Title/Abstract] OR "liver cancer"[Title/Abstract] OR "hepatic cancer"[Title/Abstract] OR "liver tumour"[Title/Abstract])
COCHRANE	Value or benefits or importance in All Text AND Diagnosis or diagnosing or diagnostic or screening in All Text AND Glypican-3 or "glypican 3" or glypican in All Text AND "Hepatocellular carcinoma" or "hepatocellular cancer" or "liver cancer" or "hepatic cancer" or "liver tumour" in All Text - in Cochrane Reviews, Cochrane Protocols, Trials, Clinical Answers, Editorials, Special Collections (Word variations have been searched)
CINAHL	AB (Value or benefits or importance) AND AB (Diagnosis or diagnosing or diagnostic or screening) AND AB (Glypican-3 or "glypican 3" or glypican) AND AB ("Hepatocellular carcinoma" or "hepatocellular cancer" or "liver cancer" or "hepatic cancer" or "liver tumour")
MEDLINE	AB (Value OR benefits OR importance) AND AB (Diagnosis OR diagnosing OR diagnostic OR screening) AND AB (Glypican-3 OR "glypican 3" OR glypican) AND AB ("Hepatocellular carcinoma" OR "hepatocellular cancer" OR "liver cancer" OR "hepatic cancer" OR "liver tumour")

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria: Major inclusion criteria include (1) studies evaluating the diagnostic utility of serum GPC-3 in HCC patients. (2) Studies where the study groups were HCC patients, whereas the control groups were individuals with benign chronic liver diseases (liver cirrhosis, liver fibrosis, hepatitis B or C virus infections, alcoholic liver disease, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis or their combination) [35]. (3) studies where sensitivity, specificity, true positive (TP), false positive (FP), true negative (TN), and false negative (FN) were recorded or can be calculated. (4) Studies reported in English. (5) studies that included only subjects over the age of 18 years. (6) Studies that included both male and female subjects.

Exclusion criteria: Major exclusion criteria include: (1) Studies not done on humans. (2) Studies that used or included healthy Individuals as control. (3) Studies that used specimens other than serum. (4) repeated or duplicated studies; in such a situation, the studies with the most recent or most detailed data reporting are chosen. (5) Studies without full text or with incomplete data for reporting. (7) studies that conducted GPC-3 measurements at the gene level and not the protein level.

Data extraction

After choosing all the relevant papers, the data was extracted. The following data were extracted: First author's name and year of study publication, country of study subjects, characteristics of the HCC group, characteristics of the control group, the number of the study subjects, assay type, cut-off value, average GPC-3 serum concentration in the HCC group, average GPC-3 serum concentration in the control group, gender distribution in the HCC group, gender distribution in control groups, the average age of the HCC group, the average age of the control groups, and finally, the raw data (the number of TP, FP, FN, and TN) were extracted or calculated. The whole data extraction process was repeated, and the extracted data were compared (the initial data extracted was

compared with the data extracted after a repeat extraction). Any noticed discrepancies were resolved to ensure the reliability and accuracy of the data. The formulas used to calculate the values of TP, FP, FN, and TN are as follows: TP = number of patients with HCC times sensitivity; FN = number of HCC patients times (1 - sensitivity); TN = number of non-HCC patients times specificity; FP = number of non-HCC patients times (1- specificity). The extracted data is shown in table 2.

Quality assessment of included studies

Quality assessment of the included studies was done with The Quality assessment of diagnostic accuracy studies 2 (QUADAS-2) tools, as recommended by Cochrane [38]. Each of the signalling questions provided to aid in assessments of risk of bias was given a "yes", "no", or "unclear" response. The items that evaluated the applicability concerns were each given a rating of "high," "low," or "unclear." The outcome of the quality assessment is depicted in figure 2.

Statistical analysis and outcome indicators

The recommendations of the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy were used [39]. The following software programmes, Meta-Disc version 1.4 [40] and Review Manager version 5.4.1 [41, 42], were used to conduct statistical analysis. Pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and the respective 95% confidence intervals were generated. The overall diagnostic sensitivity, specificity, PLR, NLR, and DOR, as well as the heterogeneity of the eligible studies, were represented using forest plots. The overall diagnostic performance of GPC-3 was shown using summary receiver operating characteristic (sROC) curves [43, 44].

Sensitivity measures the percentage of true positives among all individuals with a disease; in other words, it checks the capacity of a test method (tumour marker) to correctly identify a positive result for a diseased patient. Specificity measures the true negative rate among people without a disease; that is, the capacity of a test method to correctly identify a negative result for a non-diseased individual [45]. PLR is the probability that a positive test will be expected in a subject with a disease compared to the likelihood that a positive test will be expected in a subject without a condition. The NLR is the probability that a subject with a disease tests negative compared to the probability that a subject without a disease tests negative [45]. DOR measures the effectiveness of the test; it is an indicator that combines sensitivity and specificity. It is the ratio of the odds of a test being positive in people with a disease to the odds of a test being positive in people without a disease [46, 47]. The area under the summary receiver operating characteristic (sROC) curve is an index that evaluates the diagnostic accuracy of a test as a whole, across many studies in a meta-analysis of diagnostic test accuracy, as opposed to the diagnostic accuracy at a particular threshold [43, 48].

The between-study heterogeneity was evaluated using the X^2-based Q-statistic, and $P<0.05$ was considered statistically significant. Additionally, the inconsistency index, also known as the I-squared (I^2) (a numerical indicator of consistency across studies) [40], was computed. The heterogeneity of the obtained data was examined using the I^2-value. An I^2-value less than 50% and a P-value greater than 0.05 indicated minimal heterogeneity. If heterogeneity was not observed, the fixed-effects model was used for the meta-analysis. If heterogeneity was observed, the random-effects (DerSimonian-Laird) model was used [49]. The Spearman correlation coefficient was computed to test whether the threshold effect could explain the observed heterogeneity [40, 50]. A meta-regression analysis was used to check if other covariates could explain the heterogeneity [40, 50].

RESULTS

Study selection

A flowchart of the process of literature search and selection is shown in Figure 1. Firstly, 144 possibly relevant articles were retrieved from the databases described earlier. After that, 69 studies were found to be duplicates and were removed. Abstracts and titles of 75 remaining studies were examined. After examining the titles and abstracts, 53 studies were removed because they failed to meet the inclusion criteria of the systematic review. Subsequently, 11 papers were later dropped after reading the whole text of the remaining 22 papers. Eleven studies were dropped because the full text was not written in English in two of them, the study used healthy or normal controls in four of them, the study design was not relevant, or the data was not in a form that could be reported on in three of them, and access to the full text of the paper was limited in two of them. Finally, the remaining eleven studies were selected for systematic review and meta-analysis (Figure 1). All the selected studies documented that approval was obtained from their respective ethics committees.

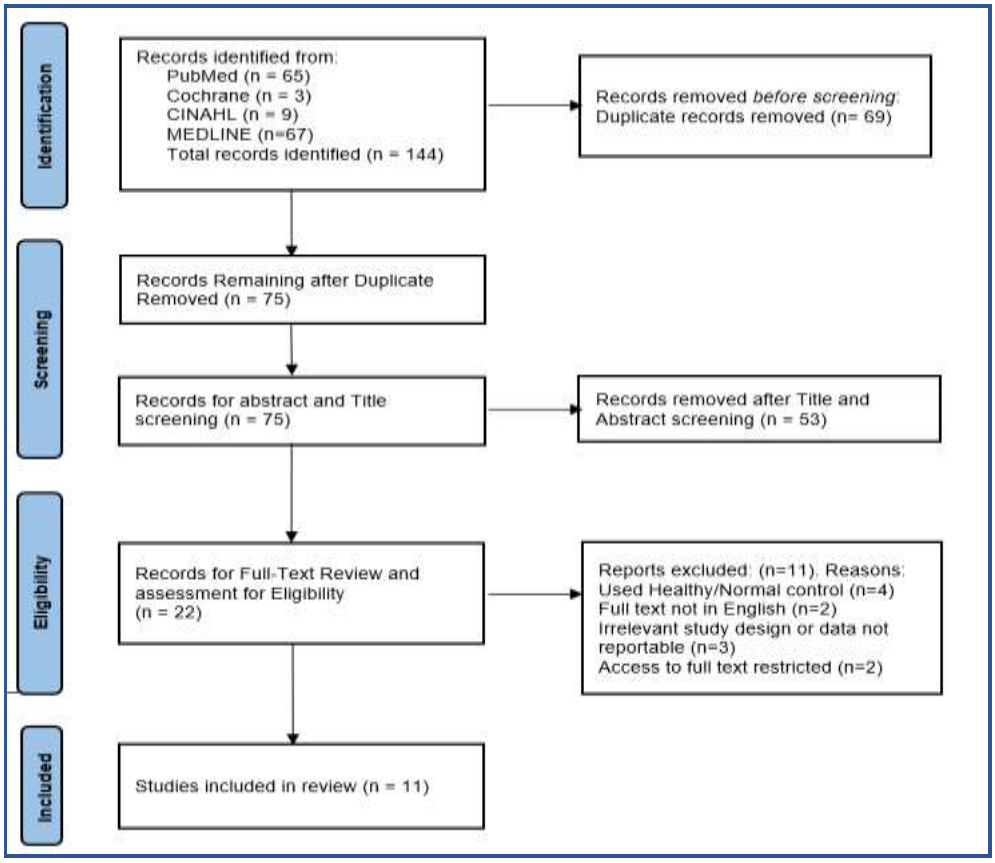


Figure 1: PRISMA flowchart showing the study selection process. PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-analyses [51]

STUDY CHARACTERISTICS

This meta-analysis assesses the diagnostic utility of serum GPC-3 in HCC in 11 case-control studies, with a total of 1169 cases of patients with HCC and 1031 controls. The selected studies in the HCC group comprise studies with HCC patients with or without Hepatitis B virus (HBV), Hepatitis C virus (HCV), liver cirrhosis, liver fibrosis, non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), and alcoholic liver disease (ALD). The control group involved patients with HBV, HCV, liver cirrhosis, liver fibrosis, NASH, NAFLD, ALD, or any combination of them. Enzyme-linked immunosorbent assay (ELISA) or chemiluminescence was used in the assay of serum GPC-3. The specific data of each trial are presented in Table 2.

Table 2: Characteristics of Included studies

Average age: HCC	Average age: Control	TP	FN	TN	FP
59.9±4.5	57.7±10.5	33	22	53	2
67(62-70)	58(49-66)	45	27	98	21
≤55yrs: n=100.	≤55yrs: n=77	116	82	99	27
60.9±9.66	56.7±8.7	17	13	21	9
67(31-890	61(33-82)	109	40	102	98
60.8 ±11.8	56.7 ± 10.8	94	63	81	75
54.9 ± 12.1	47.12±9.12 (Calculated)	83	55	61	57
59.4±10.9	49.7±8.1	65	55	26	14
63±9.9	58±12.7	46	29	23	32
58.8 ±12.7	45.95±12.26 (calculated)	53	47	99	1
55.4± 9.91	53.3±5.81	35	40	30	2

HCC: Hepatocellular carcinoma; GPC-3: Glypican-3; TP:

Characteristics of HCC	Characteristics of Controls	Number of subjects (HCC/control)	Assay type	cut-off value	Average GPC-3 value (HCC group)	Average GPC-3 value (Control group)	Gender: HCC	Gender: Contr
Patients with HCC, CHC and LC	Patients with LC and CHC	55/55	ELISA	2.0ng/ml	NR	NR	38/17	35/20
Patients with HCC and LC plus NASH/NAFLD	LC plus NASH/NAFLD	72/119	ELISA	64pg/ml	4.95 (2.87–7.03) pg/ml	2.17 (1.35–3.30) pg/ml	57/15	64/55
Patients with HCC and LC.	CHB or LC	198/126	Chemilumi	NR	0(0-10988) int	0(0-5800) int	144/54	68/58
HCV and LC patients with HCC	HCV and cirrhosis	30/30	ELISA	1.23ng/ml	0.835 (0.32-1.21) ng/ml	6.09 (0.194-150.3) ng/ml	20/10	12/18
LC and HCC of Viral aetiology	LC of viral aetiology	149/200	ELISA	73 pg/mL	144 (62–246) pg/mL	71 (37–163) pg/mL	123/26	134/66
HCC From multiple aetiology, underlying LC (in 87.3%) and CH (in 6.4%)	LC from multiple aetiology	157/156	ELISA	0.61ng/ml	0.92ng/ml (0-3.09) (Assay 1)	0.86ng/ml (0.007-7.40) (Assay 1)	127/30	90/66
Patients with HCV and HCC	HCV and Fibrosis or Cirrhosis	138/118	ELISA	6 ng/ml	10.9 ± 7.6ng/ml	5.53 ± 3.8 ng/ml (Calculated)	NR	NR
Aetiology from; HBV 62.5%, HCV 15%, Alcohol 10.8%, Others 11.7%	CLD all arising from HBV, with 50% having cirrhosis	120/40	ELISA	73ng/ml	75.8 (21.7-482.5) ng/ml	66.4 (2.33-66.4) ng/ml	94/26	32/8
Cirrhotic patients with HCC	Cirrhotic patients without HCC	75/55	ELISA	3.9 pg/ml	5.13 pg/ml (range53.9–93.2)	5.51 pg/ml (range 53.9–236.2)	54/21	33/22
HCC with: LC (n=87), HBV (n= 59), HCV (n=11), Alcohol dependency (n= 11), Undetermined (n= 13)	HBV- 50 patients. LC- 50 patients	100/100	ELISA	NR	46.3 (0–7826.6)	0 (0–43.6)	75/25	64/36
HBV in 84% of patient, HCV in 16.0%	All have LC, HBV in 78.1%, HCV in 21.9%	75/32	ELISA	0.3ng/ml	NR	NR	55/20	24/8

Study author and year	Country of subjects
Malov et al., 2021 [24]	Russia
Caviglia et al., 2021 [25]	Italy
Wu et al., 2020 [36]	China
Gomaa et al., 2020 [26]	Egypt
Caviglia et a et al., 2020 [27]	Italy
Jeon et al 2016 [52]	South Korea
Attallah et al 2016 [53]	Egypt
Lee et al., 2014 [54]	Korea
Ozkan et al., 2011 [28]	Turkey
Tangkijvanich et al 2010 [55]	Thailand
Liu et al., 2010 [56]	China

QUALITY ASSESSMENT OF THE INCLUDED STUDIES

The QUADAS-2 tool was used to evaluate the quality of the included studies, as shown in Figure 2. The qualities, however, fell short of expectations. None of the studies was randomised controlled trials (RCTs); instead, they were all retrospective in design (although some of the studies followed the control group subjects prospectively for a period to rule out concealed HCC). One study employed consecutive enrolment of all their study subjects [53]; therefore, its risk of bias for the patient selection domain was classified as "low risk". Two studies excluded eligible subjects [25, 36]; therefore, the risk of bias for their patient selection domain was classified as "high risk". The remaining studies did not fully explain how all their subjects were enrolled; as a result, the risk of bias for the patient selection domain in these groups of studies was classified as "unclear". Most studies determined the serum GPC-3 cut-off value from the ROC curve analysis; hence their threshold was not pre-specified. Two studies did not record the cut off-value of serum GPC-3 [36, 55]; therefore, their applicability concerns in the index test domain were marked "unclear". None of the included studies indicated whether blinding was employed in the interpretation of the index test. In other words, none of the studies specified whether the interpretation (reading) of the index test was made without the knowledge of the findings of the reference standard. As a result, all studies' risk of bias in the index test domain was classified as "unclear". All studies used reference standards recognised by either AASLD, EASL, or NICE [5]; hence, all risks of biases in the reference standard domain were classified as "low risk". In the flow and timing domain, the risks of bias in two studies [27, 54] were classified as "low risk" because they documented that the reference standard and index test were performed at the same time. The remaining studies were classified as "unclear" because they did not clarify whether there was an appropriate time interval between the index test and reference standard

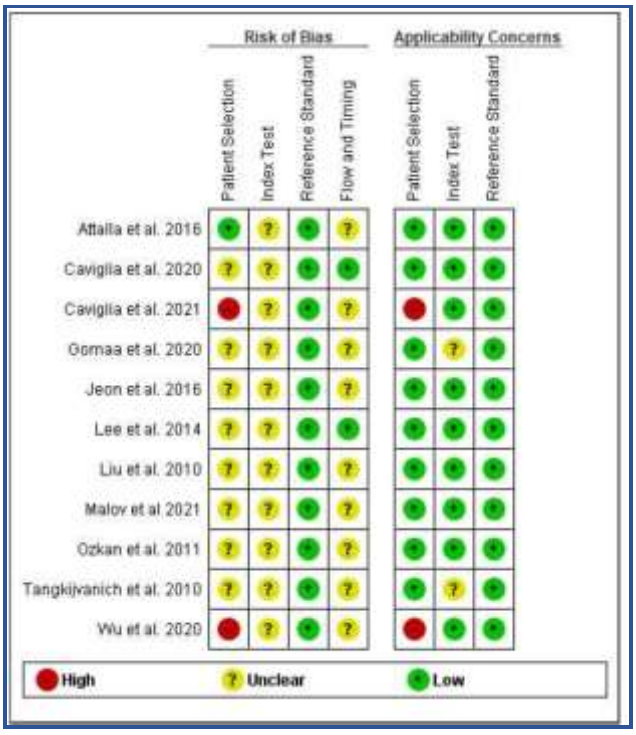


Figure 2: Summary of Quality Assessment of the included studies (Risk of bias and applicability concerns summary) BY QUADAS-2.

Diagnostic value of GPC-3 for HCC

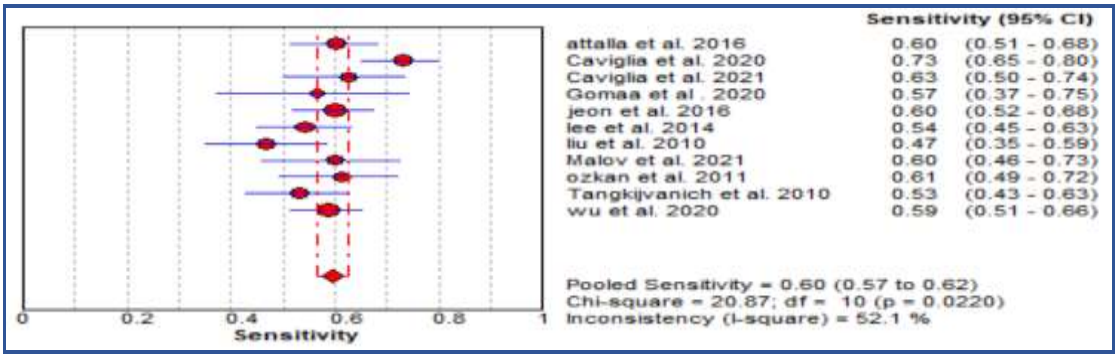
Because of the obvious heterogeneity between studies (I^2 values greater than 50% and $P < 0.05$), the random-effects model was used in this meta-analysis [49]. The sensitivity, specificity, PLR, NLR, and DOR with their respective 95% confidence intervals of each of the included studies are shown in figures 3-5. The pooled sensitivity, specificity, PLR, NLR, and DOR with corresponding 95% confidence intervals were determined. The area under the sROC curve was also determined.

Sensitivity and Specificity

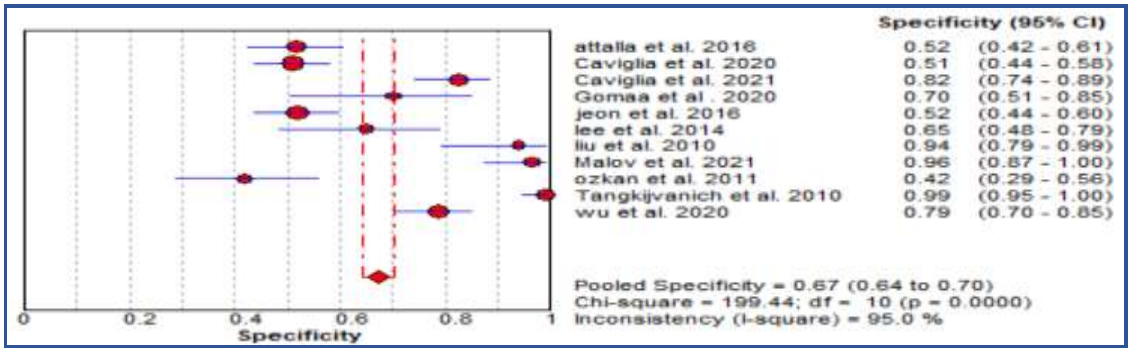
According to the meta-analytic findings, serum GPC-3 pooled overall diagnostic sensitivity and specificity in the diagnosis of HCC, with 95% confidence intervals were 0.60 (0.57-0.62) and 0.67 (0.64-0.70), respectively (Figure 3A and 3B). This means that this meta-analysis has shown that serum GPC-3 correctly identified 60% of individuals with HCC as having HCC. It also correctly identified 67% of subjects without HCC (from the control group) as not having HCC [45, 57]

Positive and Negative Likelihood ratios

The pooled overall positive and negative likelihood ratios with their 95% confidence intervals are 2.11 (1.51-2.93) and 0.59 (0.51-0.68), respectively (Figure 4). Hence, this meta-analysis has shown that the probability of correctly identifying someone with HCC is 2.11 times higher than the probability of wrongly identifying someone without HCC as

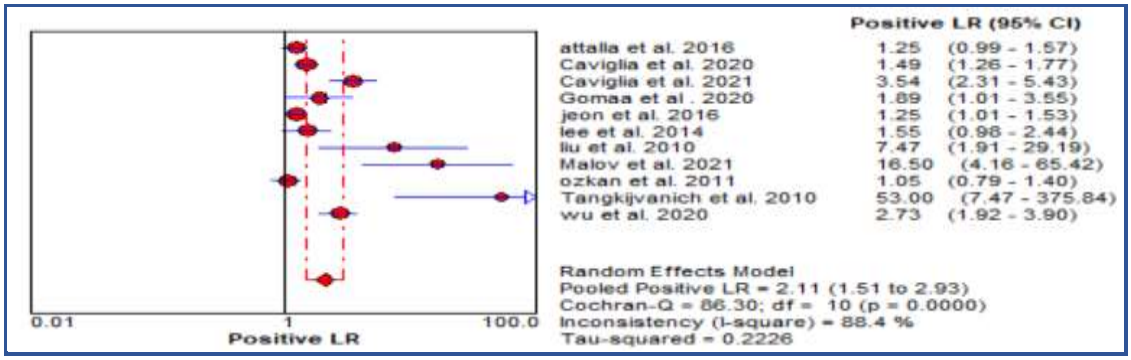


A.

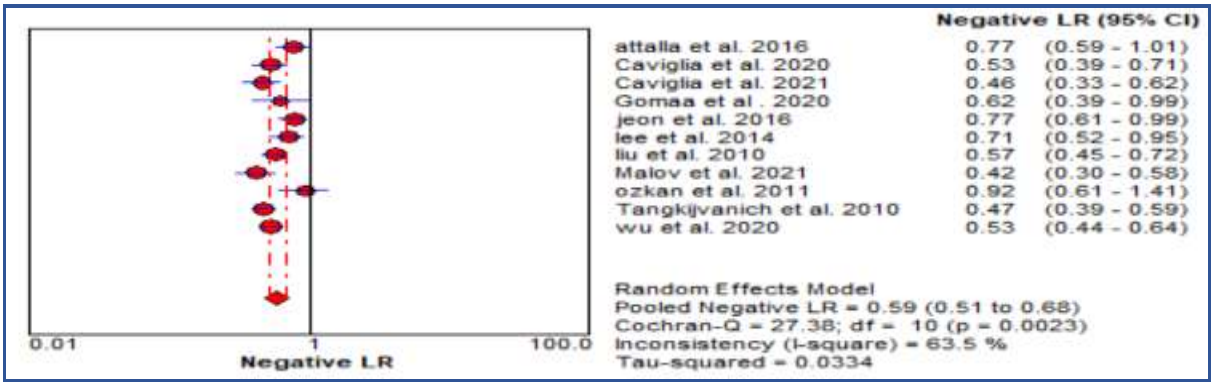


B.

Figure 3: Forest plots: (A) Showing the sensitivity of GPC-3 in HCC diagnosis. (B) Showing the specificity of serum GPC-3 in HCC diagnosis, the size of each solid circle reflects the sample size of the corresponding study.



A.



B.

Figure 4: Forest plot: (A) showing the Positive likelihood ratio of GPC-3 in HCC diagnosis. (B) showing the Negative likelihood ratio of serum GPC-3 in HCC diagnosis. The size of each solid circle reflects the sample size of the corresponding study.

Having HCC, using serum GPC-3 as a tumour marker. This meta-analysis also showed that the likelihood of an HCC patient testing negative compared to the likelihood of a non-HCC patient testing Negative is 0.59 using serum GPC-3 as a tumour marker [45].

DIAGNOSTIC ODDS RATIO

The pooled DOR and the 95% confidence intervals for serum GPC-3 in this meta-analysis were 4.07 (2.37-6.98) (Figure 5). This means that the odds of positive results among people with HCC were 4.07 times greater than the odds of positive results among subjects without HCC [47].

THE AREA UNDER THE SUMMARY RECEIVER OPERATING CHARACTERISTIC CURVE

The area under the sROC curve for GPC-3 was 0.7194 (Figure 6) in the current meta-analysis, suggesting that serum GPC-3 has a good/acceptable diagnostic accuracy for HCC [47, 58].

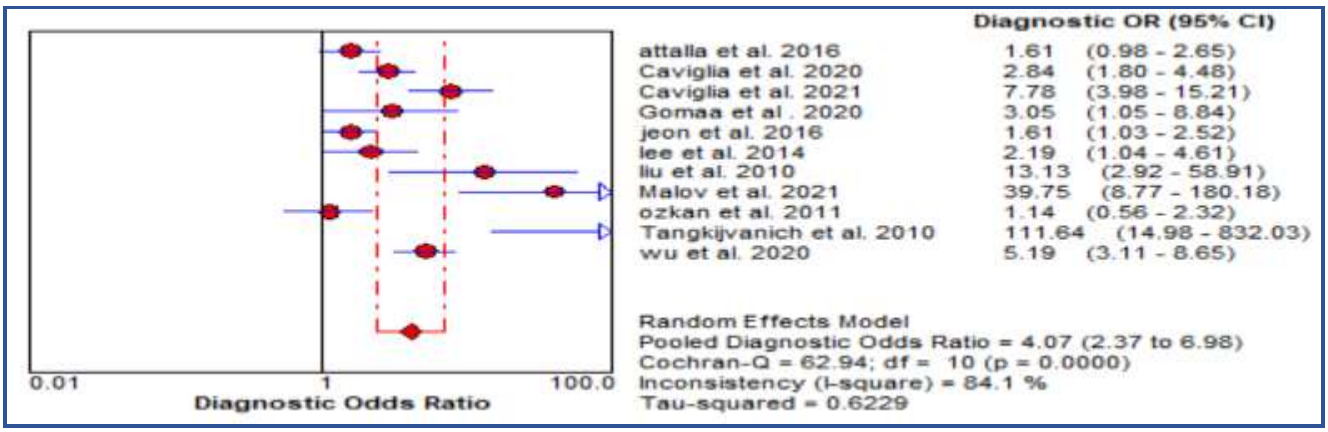


Figure 5: Forest plots of diagnostic odds ratio (DOR) of serum GPC-3 in the diagnosis of HCC.

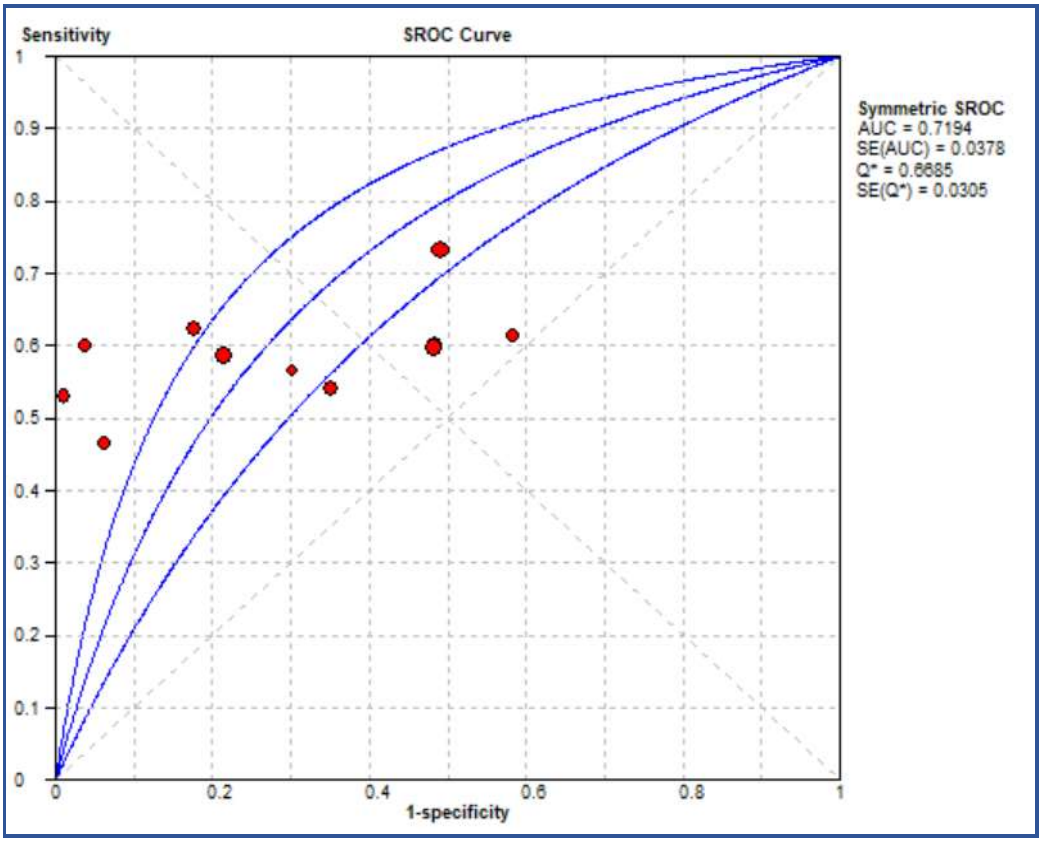


Figure 6: **Summary receiver operating characteristic curves of GPC-3 for the diagnosis of HCC, with the 95% confidence interval. Each solid circle corresponds to a study. The size of the solid circle denotes the sample size used in each study.**

TEST FOR HETEROGENEITY

Sensitivity, specificity, PLR, NLR, and DOR had respective I^2-values of 52.1%, 95.0%, 88.4%, 63.5%, and 84.1%. These findings show that there was significant heterogeneity among the studies that were eligible. The spearman technique was used to determine if the heterogeneity was accounted for by a threshold effect. The variability among the selected studies could not be justified by the threshold effect, according to the Spearman correlation coefficient between the logit of sensitivity and the logit of 1-specificity, which was 0.564 (P = 0.071) [40, 50, 59]. A meta-regression analysis was conducted to explain the heterogeneity observed among studies by analysing the study characteristics [40]. However, the assay type, sample size, and mode of enrolment (whether consecutively or not) could not explain the observed heterogeneity. Subgroup analysis using the continent of the study was done. It was noticed that the heterogeneity in sensitivity among studies done in Europe and studies done in Asia improved with an I^2-value of 45.2% and 14.0%, respectively. However, there was no significant improvement in the heterogeneity of the pooled specificity, PLR, NLR, and DOR among the studies done in both continents. Also, subgroup analysis excluding the two studies that did not report their serum GPC-3 cut-off value [36, 55] did not show significant improvement in heterogeneity. It is noteworthy that the Cochrane handbook for systematic reviews of diagnostic test accuracy recommends against investigating publication bias when there is significant heterogeneity in DOR, because the results obtained from the funnel plot will not be reliable [39, 60]. Hence, publication bias was not checked in this study.

DISCUSSION

It has been shown that cancers, when detected early, improve survival and the chances of cure [61, 62]. This is true with HCC because it is now regarded as treatable cancer as long as it is detected early [63, 64]. It has been shown that when diagnosed early, HCC's five-year survival rate is more than 70%, but when diagnosed in an advanced stage, the five-year survival rate is less than 16% [65]. HCC serum tumour markers have shown their benefit in terms of cost and convenience over histopathological testing, CT, MRI or CEUS [8]. AFP is a widely used tumour marker for HCC screening and diagnosis, but it is limited by low sensitivity of about 41-65%. And it was also found to be elevated in hepatitis and liver cirrhosis [12, 66]. Because of these, there is a pressing need to identify a novel biomarker to increase the diagnostic precision of HCC. This systematic review and meta-analysis is aimed to assess the diagnostic value of serum GPC-3 in HCC.

GPC-3, a promising potential tumour marker for HCC, has been extensively researched; it is expressed extensively during prenatal development but is repressed in most normal adult tissues. Recent research demonstrates that GPC-3 is abundantly expressed in HCC tissues and enhances cell proliferation. GPC-3 participates in WNT and the Hedgehog signalling pathways [19].

Many studies have explored the value of GPC-3 in the diagnosis of HCC [24-28, 36, 52-54, 56]. But their results have been conflicting and controversial. For example, Malov et al. (2021) [24] found that serum GPC-3 has excellent diagnostic ability in distinguishing patients with chronic Hepatitis C in the stage of liver cirrhosis from patients with Hepatitis C in the stage of liver cirrhosis and HCC; in this study, serum GPC-3 showed a sensitivity of 60% and a specificity of 96.4%. Another research by Caviglia et al. (2021) [25] showed that serum GPC-3 was also outstanding (sensitivity of 62.5% and specificity of 82.4%) in a group of patients with NAFLD/NASH in distinguishing between the ones with HCC and the ones without HCC. On the contrary, Jeon et al. (2016) [52] identified a moderate ability of GPC-3 in distinguishing HCC patients from liver cirrhosis patients, with a sensitivity of 60% and a specificity of 52%. These conflicting results are seen in other studies [28, 53]. Consequent to the numerous controversial results from different studies, the tool of meta-analysis, which was described by Crombie & Davies (2009) [67] as a logical and beneficial way of addressing practical challenges that confront anybody who is attempting to appreciate the efficacy of research, was used to analyse the diagnostic value of serum GPC-3 in HCC patients.

Firstly, the inclusion and exclusion criteria were established, and specific provisions were set for HCC and controls. Most importantly, patients with benign (non-malignant) chronic liver diseases [35] were used as the controls; this is because, according to Villanueva, (2019), HCC is rare in people without liver diseases [3]. The major risk factors of HCC are HBV, HCV, Hepatitis D virus infection, ALD, NAFLD and NASH. Up to 80-90% of HCC arises in conditions where there is underlying liver cirrhosis. [3, 68, 69]. This study did not allow investigations that merely used healthy controls or incorporated healthy people into their control groups because HCC rarely arises from healthy individuals [3]. Healthy individuals are unlikely to be routinely tested for HCC in clinical situations and hence will not constitute the right control group for HCC diagnosis. The fact that healthy controls were avoided made this study to be different from other meta-analyses that incorporated healthy individuals into their study control group [7, 33]. Thus, studies that used patients with Liver cirrhosis, liver fibrosis, hepatitis B virus infection, hepatitis C virus infections, ALD, NAFLD or their combination as controls were included in this meta-analysis.

Finally, eleven carefully chosen studies were included in this meta-analysis. It was discovered that GPC-3, with a sensitivity of 60%, specificity of 67%, PLR of 2.11, NLR of 0.59, DOR of 4.07, and the area under the sROC curve of 0.7194 has a good diagnostic value in differentiating HCC from benign chronic liver diseases. The area under the sROC curve is considered "good" or "acceptable" when it is between 0.7-0.8 [47, 58]. Hence, GPC-3, which showed an area under the sROC curve of 0.7194 in this study, has an acceptable diagnostic value in diagnosing HCC as compared to benign chronic liver diseases.

The sensitivity, specificity, and area under the sROC Curve of GPC-3 obtained in the current meta-analysis (though similar) were different from those reported in previous meta-analyses [7, 29-34]. For example, Xu et al. (2019) reported that serum GPC-3 has a sensitivity of 0.55, specificity of 0.58 and an area under the sROC curve of 0.7795 in diagnosing HCC compared to liver cirrhosis. This disparity might be explained by the number of included studies or by the different characteristics of the HCC and control groups. It might also be explained by the use of various detection reagents, detection techniques, and equipment from various countries.

There was a lot of heterogeneity among the included studies; because of this, the random effect model was used in this meta-analysis [49]. The sources of heterogeneity were investigated. A Spearman correlation analysis was performed to investigate whether the wide variations in cut-off values of included studies could explain the heterogeneity. The results demonstrated that there was no threshold effect in this study. Other variables may have contributed to the observed heterogeneity; therefore, meta-regression analysis was performed, and it was discovered that heterogeneity was not explained by GPC-3's assay type, sample size, and mode of enrolment of the study population. To further investigate the source of heterogeneity, a subgroup analysis was performed. It was noticed that the heterogeneity in sensitivity among studies done in Europe and among studies done in Asia improved with an I^2-value of 45.2% and 14.0%, respectively. However, this improvement was not noticed in other parameters of diagnostic test accuracy in these two continental groups. Also, when the two studies that did not report serum GPC-3 cut-off values [36, 55] were removed and subgroup analysis was done, no improvement in heterogeneity was observed. Differing study populations, non-explicit inclusion and exclusion criteria, and varying tumour burdens among the included studies might be the roots of the problem.

Publication bias was not checked using a funnel plot because, according to the Cochrane handbook for systematic reviews of diagnostic test accuracy, even the most acceptable method (Deeks test) of detecting publication bias has a "particularly low power" when heterogeneity in DOR is present [39]. The assertion was corroborated by Deeks et al. (2005) [60]. Hence, performing a funnel

plot of asymmetry in this study will not produce a reliable result since the DOR of this meta-analysis showed substantial heterogeneity with an I^2-value of 84.1%.

To my knowledge, this current study is the only systematic review and meta-analysis on the diagnostic value of serum GPC-3 in HCC patients produced after 2019. Therefore, it captured the most recent studies published between 2019 and the date of the literature search. (Studies that were not captured in other meta-analyses) [24-27, 36]. Thus, this meta-analysis captured the most recent evidence on the diagnostic value of GPC-3 in diagnosing HCC.

This study has some limitations. The included studies are few, which resulted from meticulous inclusion and exclusion criteria and the need to include only studies that didn't incorporate healthy controls. The small number of participants may have led to the witnessed heterogeneity [70]. The quality of the included studies was not satisfactory. All the studies were retrospective in design. There were no explicit inclusion and exclusion criteria as HCCs resulting from different aetiologies were included, while the control groups were comprised of different types of chronic liver diseases. Only one study employed consecutive enrolment of all their subjects [53]. None of the included studies specified if blinding was done in the interpretation of the index test and reference standard results. None of the included studies accounted for patient withdrawal or invalid specimens. This study did not include populations from all racial groups; only populations from Europe, Asia, and North Africa were included. Populations from North America, South America, and sub-Saharan Africa were not included. The study was not designed to delineate the diagnostic value of GPC-3 in early and advanced stages of HCC, as both early and advanced HCC patients were incorporated in this study. Some of the included studies reported that serum GPC-3 was stored in a frozen form. Long-term storage of GPC-3 may affect its stability, thus affecting diagnostic performance [71]. However, it is uncertain if the diagnostic ability of GPC-3 will be significantly impacted when serum samples are frozen for an extended period (no study on this was found).

Therefore, the following modifications might increase the quality of the study and minimise heterogeneity: (1) To fully establish the clinical usefulness of serum GPC-3 in the diagnosis of HCC, it will be necessary to conduct larger multicentre trials involving bigger cohorts over a longer period of time. (2) Well-designed double-blind studies with consecutive or random patient enrolment should be planned to prevent bias. (3) The patient cohorts for liver cirrhosis, chronic hepatitis, NASH, NAFLD, ALD, and others can be divided into subgroups, and the diagnostic utility of GPC-3 investigated in them. The patient cohorts can also be arranged into subgroups based on tumour site, size, and stage. (4) Future studies should incorporate all continents and races (including North Americans, South Americans, and Sub-Saharan Africans) to explore the impact of race, continent, and country of origin on the diagnostic properties of serum GPC-3. (5) Future studies can investigate how serum GPC-3 compares with other tumour markers in the diagnosis of HCC and how serum GPC-3 can complement other diagnostic markers in diagnosing HCC.

CONCLUSION

This systematic review and meta-analysis showed that serum GPC-3 has an acceptable value as a diagnostic marker for diagnosing patients with HCC compared to patients with benign chronic liver diseases. This finding is reassuring because it has shown that serum GPC-3 has the potential to give diagnostic value in addition to that offered by other diagnostic protocols for HCC. However, this study has some limitations, including the low quality of included studies, a small number of included studies, and heterogeneity among included studies. It is suggested that in the future, well-designed, large-sample prospective studies should be done to objectively look at the diagnostic accuracy of GPC-3 in people with HCC and figure out if it might be more useful than other biomarkers that are routinely used. It is also recommended that more research should be done on the diagnostic value of serum GPC-3 in different stages of HCC and in HCC caused by various aetiological factors.

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