International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2024; 7(3): 24-29
Received: 19-04-2024
Accepted: 24-05-2024

Alaa Mohammed Ramadan
Clinical Pathology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

Osama Alsaid Negm
Tropical Medicine Department, Faculty of Medicine, Tanta University, Tanta, Egypt

Wesam Salah Mohamed
Clinical Pathology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

Amal Said Bendary
Clinical Pathology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

Corresponding Author:
Alaa Mohammed Ramadan
Clinical Pathology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

Study of vimentin serum level and gene methylation in patients with hepatocellular carcinoma

Alaa Mohammed Ramadan, Osama Alsaid Negm, Wesam Salah Mohamed and Amal Said Bendary

DOI: https://doi.org/10.33545/pathol.2024.v7.i3a.580

Abstract
Background: The Vimentin protein (VIM) is highly prevalent among the proteins belonging to the type III IF protein family, which is present in mesenchymal cells. The purpose of this study was to assess the role of vimentin concentration in serum and methylation of vimentin gene in detecting hepatocellular carcinoma (HCC).
Methods: This current work had been performed on 60 subjects which assigned into two groups [Group I: involved 40 cirrhotic individuals with HCC, classified into two equal subgroups as follows (Group I A: with metastatic HCC and Group I B: 20 HCC patients without metastasis), Group (II): involved 40 cirrhotic individuals and without HCC] and 20 healthy subjects as control.
Results: A significant positive association existed between serum vimentin and alpha fetoprotein (AFP) in the two groups IA and IB ($p<0.05$). Serum vimentin demonstrated significant positive correlations with total serum bilirubin in Group IA and with white blood cell count in Group IB. Vimentin has a greater sensitivity (88%) and specificity (85%) than AFP. Serum vimentin had been greater in Group IA contrasted to Group IB, and in Group IB compared to Group II. The least serum level was in Group III ($P=0.001$). The methylation status of vimentin gene in HCC individuals had been significantly decreased than in individuals with cirrhosis and in Group IA than in Group IB.
Conclusions: Vimentin can serve as an additional biomarker for identifying HCC and has a crucial function in the progress and development of HCC. The measurement of serum vimentin level is a highly accurate and precise biomarker for diagnosing HCC and detection of HCC metastasis than the traditional marker, AFP.

Keywords: Vimentin, gene methylation, hepatocellular carcinoma, cirrhosis, alpha fetoprotein

Introduction
HCC ranks as the 5th most prevalent form of cancer globally and is the 3rd highest contributor to cancer-related mortality. It is detected that there are around 1 million new instances of HCC reported each year [1].
The rise in the occurrence of HCC in Egypt can be attributed to several factors. These include the growing frequency of risk factors like the emergence of HCV during the same time period, the impact of HBV infections, advancements in screening programs and tools for diagnosis, and the improved rate of survival among those suffering from cirrhosis, which allows for the development of HCC in some cases [2, 3].
Intermediate filaments (IFs) are prominent protein constituents of the eukaryotic cells' cytoskeleton [4]. Proteins belonging to the IF family are present in the cells' nucleuses, where they create a compact network resembling a mesh. This network serves to preserve the cell structural integrity and perform biochemical tasks at the interface between the cytoskeleton and chromatin [5].
Vimentin protein (VIM) is highly prevalent within the type III IF protein family and is observed in mesenchymal cells. Additionally, proteins known as IF-associated proteins (IFAPs) that arrange IFs into clusters and interconnected structures were observed. The IFAPs facilitate the coordination of contacts between IFs and other components of the cytoskeleton, as well as organelles. IFs and IFAPs function as regulators of cytoplasmic organization in cells, contributing to the structural integrity and stability of different organs [6]. VIM expression in adults is restricted to mesenchymal cells in connective tissue, muscle and CNS.

As a result, vim is frequently utilized as an indicator of cells that originate from mesenchyme or are undergoing an epithelial-to-mesenchymal transition (EMT) in both regular development and the spread of cancers [7, 8].

Furthermore, prior research has shown that VIM is present in the circulating tumor cells (CTCs) of individuals with breast and advanced cancer of the prostate [9]. Nevertheless, it is still uncertain if EMT-related indicators are present in CTCs or has an impact in the progress of HCC [10].

It could be highly intriguing to investigate if EMT happens in HCC tissue and to establish a correlation between EMT occurrence and the production of CTCs, as EMT promotes the development of a subset of tumor cells into more aggressive phenotypes. Furthermore, it is crucial to examine if EMT-correlated markers are present in CTCs and if their levels of expression might be used as prognostic indicators among those with HCC [10].

The purpose of this work was to assess the role of vimentin concentration in serum and methylation of vimentin gene in detecting HCC.

Patients and Methods

This case-control work had been performed on 60 cirrhotic participants with HCC in comparison with cirrhotic patients without HCC and 20 healthy subjects as control. The study was done from 2016 to 2022 following permission from the Ethics Committee in Tanta University Hospitals, Tanta, Egypt. All subjects submitted a well-informed written consent.

Criteria for exclusion were chronic and acute infections rather than HCV and HBV infections, chronic illness (collagen diseases), respiratory, cardiac, or renal illnesses, chronic alcoholism, additional malignancies, and patients who had received anti-viral or systemic anti-cancer therapy; patients had undergone loco regional therapy for HCC that included percutaneous ethanol injection, ablation with radiofrequency, or trans catheter arterial embolization prior to the trial.

Subjects had been assigned into three groups: Group I: involved 40 cirrhotic individuals with HCC, classified into two equal subgroups as follows: [Group I A: with metastatic HCC and Group I B: 20 HCC patients without metastasis], Group (II): involved 20 cirrhotic individuals and without HCC and Group (III): Included 20 healthy participants with no evidence of liver disease (control group).

Each subject had been exposed to taking of history, clinical examination, laboratory tests [full blood picture (CBC), liver function tests (serum alanine aminotransferase (ALT) activity, serum aspartate aminotransferase (AST) activity, total and direct serum bilirubin level, serum albumin level and prothrombin activity), hepatitis virus markers (HCV antibody and HbsAg) and alpha fetoprotein test (AFP)], research laboratory investigations: [Determination of serum Vimentin levels by ELISA, DNA extraction from serum samples, Bisulfite modification of purified DNA and Methylation Specific real-time PCR].

Additional materials needed, but not included, are an ELISA Reader, 37 °C incubator, and pipettes with disposable tips, disposable tubes, distilled water, and absorbent paper.

Determination of serum Vimentin levels by ELISA

1. Blank well: only Chromogen solution A and B, as well as stop solution, had been permitted.
2. Standard wells: 50 ul of standard and 50 ul of Streptavidin-HRP were applied. The standard contains biotin antibodies, so there is no need to add additional antibodies.
3. The test wells: 40 ul of sample, followed by 10 ul of VIM-antibody and 50 ul of Streptavidin-HRP, were applied. The plate was coated with the sealing membrane and thereafter shaken gently. It was then incubated for 60 mins at 37 °C.
4. The 30-fold washing concentration was diluted with distilled water. The membrane was delicately removed, and the liquid was emptied. Then 0.35 ml diluted washing solution was used for wash, which is repeated 5 times.50ul chromogen solution A, Next, 50 ul of chromogen solution B were introduced into each well. The mixture was gently shaken and left to incubate for 10 minutes in a light-free environment.
5. Each well was treated with 50ul Stop Solution to promptly halt the reaction, resulting in an abrupt color shift from blue to yellow.
6. Consider blank as zero and determine the the optical density (OD) at 450 nm within 15 minutes after applying the stop solution.

DNA extraction from serum samples

QIAamp DNA Blood Mini Kit for DNA Extraction from serum.

Determination of Concentration, Yield, Purity of DNA

DNA yields were calculated based on the concentration of the DNA in the eluate, which was quantified using absorbance at 260 nm. The purity was assessed by computing the ratio of absorbance at 260 nm to at 280 nm. The A260/A280 ratio of pure DNA ranges from 1.7 to 1.9.

Bisulfite modification of purified DNA

DNA was thawed, the Bisulfite solution was checked to ensure that it is dissolved completely. The components for the bisulfite reactions were produced in 200 µl PCR tubes. The PCR tubes were sealed and the bisulfite reactions were well mixed. The tubes were kept at an ambient temperature ranging from 15 to 25 degrees Celsius. The process of converting DNA to bisulfite form was carried out utilizing a thermal cycler. The whole cycle lasted around 30 minutes. The PCR tubes underwent a short centrifugation process, after which the whole bisulfite reactions were transferred to new 1.5 ml micro centrifuge tubes. Each sample was treated with 250 ul of ethanol (96-100%), mixed by pulsating vortexing for 15 seconds, and then quickly centrifuged to eliminate any droplets on the inside of the lid. Arrange the appropriate quantity of Minelute DNA spin columns (SC) and collecting tubes in a suitable rack. Transfer the whole mixture from all the tubes into the matching Minelute DNA SC. The SC were exposed to centrifugation at the highest speed for a of 1-minute duration. The rejected flow-through was discarded, and the SC were reinserted into the collecting tubes. Each SC was treated with 500 µl of Buffer BW (wash buffer) and then centrifuged at maximum speed for 1 minute. The liquid that passed through was dumped, and the cylindrical filters were returned to the tubes used for collecting the liquid. Each SC was treated with 500 µl of Buffer BD (Desulfonation buffer) and kept at ambient temperature for 15 minutes. The SC were subjected to centrifugation at the highest speed for a duration of 1 minute. The liquid that passed through the columns was discarded, and the columns were thereafter returned to the collecting tubes. Each spin column was supplemented with

500 µl of Buffer BW and then subjected to centrifugation at the highest speed for 1 minute. The liquid that passed-through was wasted, and the columns used for spinning were returned to the tubes for collection. Once again, 500 µl of Buffer BW was introduced into each spin column and subjected to centrifugation at the highest speed for a duration of 1 minute. The liquid that passed through was disposed of, and the SC were returned to the tubes used for collecting the liquid. Each spin column was treated with 250ul of ethanol (96-100%) and then centrifuged at the highest speed for 1 minute. The SC were inserted into fresh 2 ml collection tubes and subjected to centrifugation at the highest speed for 1 minute to eliminate any remaining liquid. The SC were inserted into sterile 1.5 ml micro centrifuge tubes. A volume of 15 µl of Buffer EB (Elution Buffer) was applied to the middle of each membrane, and the lids were closed softly. The SC were left to incubate at room temperature for a 1-minute duration. The SC were exposed to centrifugation with a force of 15,000 times the acceleration due to gravity for a 1-minute duration in order to extract the DNA.

Methylation Specific real-time PCR

The PCR was conducted utilizing the DyNAmo Flash SYBR Green qPCR Kit supplied by Thermo Fisher Scientific, Inc. The package was transported using dry ice. Upon receipt, all kit components had been kept at a temperature of -20°C. The product comprises a thermophiles DNA polymerase derived from Thermus brockianus, which has been engineered to possess a hot-start capability.

Q-PCR Master Mix

The product incorporates a hot-start variant of a genetically altered Thermus brockianus DNA polymerase. The adjusted polymerase has a DNA binding domain that is not particular to any specific DNA sequence. This domain enhances the physical stability of the polymerase-DNA complex. The Tbr polymerase has been chemically changed to be non-functional at normal room temperature. This inactivation halts the elongation of primers that have bound to non-specific regions throughout the initialization of the reaction, therefore enhancing the specificity of the PCR process. The reaction may be conducted at ambient temperature. The first denaturation step in the PCR technique serves to restart the polymerase enzyme, often known as hot start.

Melting Curve

It assesses the specificity of an amplified product. As the temperature raised gradually, there was a noticeable decline in SYBR Green fluorescence as the product underwent denaturation. The Tm, or melting temperature of the product, is determined by plotting the reduction in SYBR Green fluorescence as a negative first derivative throughout the temperature rise and identifying the point at which the peak occurs. The distinction between particular and generic goods was based on the disparity in their melting temperatures. Should primer-dimers or other nonspecific products be detected, it is necessary to evaluate the efficiency of the PCR. Inconsistent effectiveness results in inaccurate measurement.

Relative Quantification

It has been employed to ascertain the ratio between the amount of a certain molecule in the specimen and in the calibrator (a reference gene).

Statistical analysis

Statistical analysis had been conducted utilizing SPSS v26 (IBM Inc., Chicago, IL, USA). Quantitative parameters had been displayed as mean and standard deviation (SD) and contrasted among all groups utilizing ANOVA (F) test with post hoc test (Turkey). Qualitative parameters had been displayed as frequencies and percentages (%) and parameters had been analysed employing the Chi-square test. Pearson moment correlation equation for linear relation of normally distributed variables and Spearman rank correlation equation for non-normal variables/non-linear monotonic relation. Evaluation of diagnostic performance specificity, sensitivity, negative predictive value (NPV) and positive predictive value (PPV). A two tailed P value < 0.05 was considered statistically significant.

Results:

Demographic data demonstrated non-significant variation among the two groups. Table 1.

Table 1: Comparison between the studied groups as regards demographic data

		Group I A (N=20)	Group I B (N=20)	Group II (N=20)	Group III (N=20)	P
Age		60.95±8.57	61.50±7.11	61.20±8.91	60.25±10.95	0.976
Sex	Male	16(80.0%)	13(65.0%)	15(75.0%)	10(50.0%)	0.188
	Female	4(20.0%)	7(35.0%)	5(25.0%)	10(50.0%)	

Data are presented as mean± SD or frequency (%). P1: Group I A, and Group I B, P2: Group I A, and Group II, P3: Group I A, and Group III, P4: Group I B, and Group II, P5: Group I B and Group III, P6: Group II and Group III.

Hb concentration and PLT Count showed significant reduction in groups I and II contrasted to the control group (p-value=0.001). WBCs Comparison between different groups and the control group demonstrated no statistical significance (P=0.673). AL, AST, and bilirubin revealed higher values in all groups contrasted to the control group (P=0.001). Albumin revealed significant reduction in the diseased groups, in contrast to the control group (P=0.001). INR showed significantly greater in the diseased groups contrasted to the control group (P=0.001). Table 2.

Data are presented as mean ± SD. *significant p-value < 0.05. P1: Group I A and Group I B, P2: Group I A and Group II, P3: Group I A and Group III, P4: Group I B and Group II, P5: Group I B and Group III, P6: Group II and Group III, Hb: hemoglobin, CBC: Complete Blood Count, PLT: Platelets, WBCs: White Blood Cell, AST: Aspartate Aminotransferase, ALT: Alanine Transaminase, ALB: Albumin, INR: International Normalized Ratio, AFP: Alpha Fetoprotein. AFP exhibited a significant elevation in Group IA contrasted to Group IB, and both were greater than Group II, and the lowest expression was in the control group (P=0.001). Serum vimentins were greater in Group IA contrasted to Group IB, and in Group IB contrasted to Group II. The least serum level was in Group III (P=0.001). The Methylation status of VIM gene in HCC individuals was significantly decreased in contrast to in Cirrhotic individuals. And it is decreased in Group IA contrasted to in Group IB. The highest Methylation level was in Control Group (P=0.013). There were 90% positive HCV cases in group IA, contrasted to 85% in group IB and 80% in group II, making a significant variation (P=0.001) no significant variation was existed among HbsAg values among the studied groups (P=0.249). Table 3.

Table 2: CBC and liver function tests in the studied groups

	Group I A (N=20)	Group I B (N=20)	Group II (N=20)	Group III (N=20)	P
CBC					
Hb	9.71±0.71	10.86±1.78	10.47±1.63	12.34±1.01	0.001*
	P1=0.009*, P2=0.082, P3=0.001*, P4=0.359, P5=0.001*, P6=0.001*				
PLT	111.85±47.15	132.85±100.03	140.30±78.49	251.90±75.46	0.001*
	P1=0.395, P2=0.250, P3=0.001*, P4=0.762, P5=0.001*, P6=0.001*				
WBCs	5.60±4.02	6.71±3.86	6.46±3.51	6.79±1.77	0.673
Liver Function Tests					
AST (U/L)	58.80±60.79	82.85±50.64	47.85±28.39	21.00±8.01	0.001*
	P1=0.076, P2=0.415, P3=0.006*, P4=0.011*, P5=0.001*, P6=0.048*				
ALT (U/L)	65.30±57.80	46.85±38.67	29.60±20.09	20.15±9.24	0.001*
	P1=0.114, P2=0.003*, P3=0.001*, P4=0.139, P5=0.023*, P6=0.415				
ALB (g/dl)	2.44±0.54	3.08±0.56	2.97±0.56	4.29±0.42	0.001*
	P1=0.001*, P2=0.002*, P3=0.001*, P4=0.529, P5=0.001*, P6=0.001*				
INR	1.70±0.41	1.37±0.42	1.74±0.64	0.90±0.18	0.001*
	P1=0.022*, P2=0.785, P3 =0.001*, P4=0.011*, P5=0.001*, P6=0.001*				
Bilirubin (mg/dl)	1.67±0.50	3.96±3.13	3.69±2.15	0.94±0.09	0.001*
	P1=0.001*, P2=0.001*, P3=0.245, P4 0.665, P5=0.001*, P6=0.001*				

Table 3: Comparison between all groups concerning AFP, serum vimentin level, methylation status of vimentin gene, HCV and HbsAg

		Group I A (N=20)	Group I B (N=20)	Group II (N=20)	Group III (N=20)	P
AFP (ng/ml)		1817.75±1149.33	1416.37±1328.27	92.77±69.86	2.73±1.80	0.001*
		P1=0.157, P2=0.001*, P3=0.001*, P4=0.001*, P5=0.001*, P6=0.750				
Serum vimentin level		1982.75±722.68	1177.65±499.19	903.75±394.17	365.20±215.71	0.001*
		P1=0.001*, P2=0.001*, P3=0.001*, P4=0.083, P5=0.001*, P6=0.001*				
Methylation of Vimentin Gene						
Methylated		7(35.0%)	10(50.0%)	12(60.0%)	17(85.0%)	0.013*
Unmethylated		13(65.0%)	10(50.0%)	8(40.0%)	3(15.0%)	
HCV Antibodies	+ve	18(90.0%)	17(85.0%)	16(80.0%)	0(0.0%)	0.001*
	-ve	2(10.0%)	3(15.0%)	4(20.0%)	20(100.0%)	
HBs Ag	+ve	3(15.0%)	3(15.0%)	4(20.0%)	0(0.0%)	0.249
	-ve	17(85.0%)	17(85.0%)	16(80.0%)	20(100.0%)	

Data are presented as mean ± SD or frequency (%). *Significant p-value < 0.05. P1: Group I A and Group I B, P2: Group I A and Group II, P3: Group I A and Group III, P4: Group I B and Group II, P5: Group I B and Group III, P6: Group II and Group III, AFP: Alpha fetoprotein, HCV: hepatitis C virus, HBs Ag: Hepatitis B surface antigen.

Significant positive associations were existed between serum vimentin and AFP in both groups IA and IB (P=0.001 and 0.019 respectively). Serum vimentin showed significant positive correlations with total serum bilirubin in Group IA and with WBCs count in Group IB. Table 4.

Table 4: Correlations between serum vimentin level and other parameters

Vimentin	Group IA		Group IB	
	R	P-Value	R	P-Value
AFP	0.816	0.001*	0.518	0.019*
Age	0.225	0.339	0.385	0.093
Hb	0.308	0.186	0.010	0.967
PLT	0.228	0.333	0.442	0.051
WBCs	-0.105	0.660	0.519	0.019*
AST	-0.086	0.720	0.012	0.958
ALT	-0.057	0.810	0.113	0.636
ALB	-0.001	0.996	-0.185	0.434
INR	0.204	0.388	0.208	0.380
Bil	0.618	0.004*	0.025	0.915

RS: Pearson coefficients. Significant p-value < 0.05. P1: Group I A and Group I B, P2: Group I A and Group II, P3: Group I A and Group III, P4: Group I B and Group II, P5: Group I B and Group III, P6: Group II and Group III, CBC: complete blood count, Hb: hemoglobin, PLT: platelets, WBCs: white blood cell, AST: aspartate aminotransferase, ALT: alanine transaminase, ALB: Albumin, INR: international normalized ratio, AFP: Alpha fetoprotein.

Vimentin has a greater specificity (85%) and sensitivity (88%) contrasted to AFP. Combined Vimentin and AFP have a greater specificity and sensitivity contrasted to each parameter alone. Figure 1.

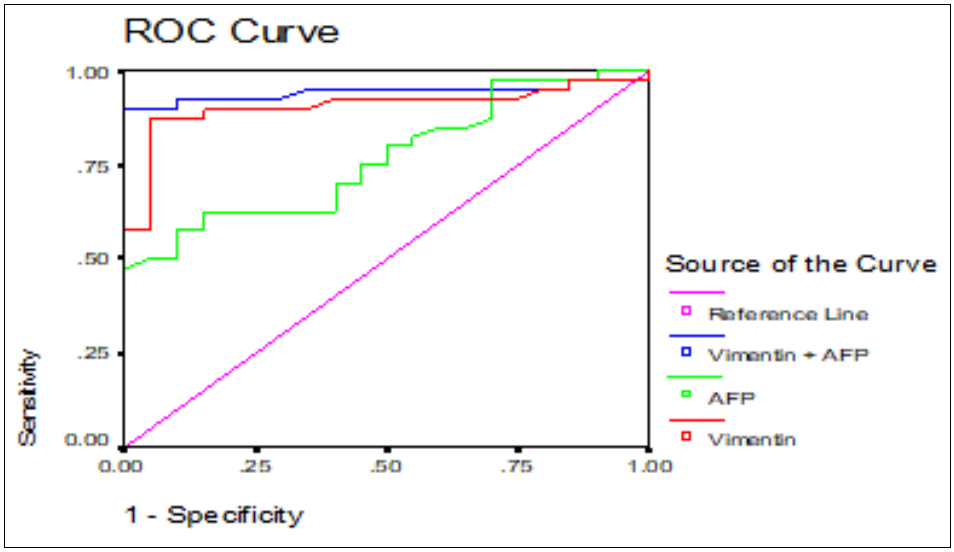

Fig 1: ROC Curve for serum vimentin, AFP and combined vimentin and AFP

Discussion

Liver cancer continues to be a significant global health issue, with its occurrence increasing on a global scale [11]. By 2025, cancer of the liver is projected to impact over 1 million people per year [12].

In this study, ELISA tool had been utilised to assess the concentration of VIM in serum. The mean concentration of vimentin in serum was significantly elevated among individuals with HCC (group I) contrasted to non-HCC cirrhotic patients (groups II) and healthy controls (group III). Also, a significant increase rise the mean serum vimentin concentration was existed in metastatic HCC individuals (group IA) contrasted with patients without metastasis (group IB). In HCC patients (group I), no statistically significant associations existed among vimentin level in serum and patient age, serum levels of ALT, AST.

The ROC curve analysis conducted to assess the diagnostic performance of serum vimentin level in comparison with AFP level for HCC diagnosis; revealed that, the sensitivity of vimentin in detecting HCC is 88%, while its specificity is 85%. While, AFP showed a sensitivity of 80% and a specificity of 75%. When combined, their specificity and sensitivity were 90% and 93% correspondingly. In line with our work, Sun *et al*. [13] revealed that Individuals with HCC of all sizes have detectable levels of soluble VIM in their bloodstream. The serum level of the tiny tumors was markedly elevated compared to the nonneoplastic controls. The research found that an increased amount of circulating vimentin may accurately identify tiny HCC measuring less than 2 cm, with a sensitivity of 40.91% and a specificity of 87.50%. Furthermore, their study showed serum vimentin was more specific and sensitive in detecting small HCC than AFP (sensitivity 16.28% and specificity 85.19%). Also, when vimentin was combined with AFP, The detection specificity and sensitivity were significantly improved to 98.15% and 58.77%, correspondingly [13]. Also, Hu *et al*. [14] showed that over-expression of VIM was significantly correlated with metastasis of HCC. This indicates that the excessive expression of VIM likely has a significant impact on the spread of HCC to other parts of the body, may be due to the role of vimentin in increasing migration, motility, and invasive potential of tumour cells [15].

The study indicated that the Methylation status of VIM gene in HCC individuals was significantly decreased in contrast to than in Cirrhotic individuals. And it is decreased in Group IA contrasted to in Group IB. The lowest Methylation level was in Control Group. In line with our work, Kitamura *et al*. [16] revealed that abnormally high levels of methylation of vimentin were seen in 56% of primary HCC cases. Curiously, the amount of vimentin methylation DNA in the HCC was often lower than in the surrounding tissues of the liver. The resected background liver tissue exhibited pathological evidence of liver cirrhosis or chronic hepatitis, indicating that the existence of chronic hepatitis virus infection or variations in inflammation could affect the occurrence of methylation events that in turn are correlated with an elevated risk of cancer of the liver. However, Idriss *et al*. [17] revealed that an insignificant correlation was existed among vimentin concentration in serum and AFP.

According to our study, comparison between the specificity and sensitivity of serum vimentin, serum AFP and combined vimentin and AFP as markers for the HCC diagnosis. Vimentin has a greater sensitivity and specificity contrasted to AFP. Combined Vimentin and AFP have a higher sensitivity and specificity than each parameter alone. In line with our work, Sun *et al*. [13] proved that vimentin was shown to be substantially elevated in HCC, specifically in the small-size sub-group (< 2 cm), with a p-value of less than 0.01. Additional examination indicated that an increased amount of vimentin in the bloodstream may accurately identify small HCC with a sensitivity of 40.91% and a specificity of 87.5%. In addition, vimentin was shown to be more effective than serum AFP tested at various thresholds in diagnosing tiny tumors. When AFP is added with it, the detection specificity and sensitivity may be increased to 98.15% and 58.77% correspondingly.

Limitations of this research was the very small sample size. The research was conducted in a solitary center. Therefore, we suggest that Vimentin might serve as a diagnostic tool for detecting HCC. Vimentin has the capacity to function as a tool for diagnosis for detecting HCC.

Conclusions

Vimentin could be utilized as an additional biomarker in detecting HCC, additionally, it plays a crucial function in the progress and advancement of HCC. The invasion and migration of tumour cells are the direct results of excessive expression of vimentin in these cells. Numerous studies

have consistently shown a strong correlation between vimentin over-expression and the development of an invasive phenotype. The concentration vimentin in serum as a biomarker for diagnosing HCC and detecting HCC metastasis exhibited more specificity and sensitivity compared to the standard marker, AFP. The combination of AFP and vimentin revealed enhanced specificity and sensitivity in the detection of HCC. Thus, VIM may serve as a valuable supplementary biomarker, together with AFP, for the detection of HCC in individuals with hepatic cirrhosis.

Financial support and sponsorship: Nil

Conflict of Interest: Nil

References

1. Theise N. Liver and Gallbladder. In: Kumar V, Abbas AK, Aster JC, Editors. Robbins & Cotran *pathologic basis of disease*. 9th Ed. Lincoln, Lincolnshire, United Kingdom: Saunders Elsevier; c2003. p. 821-82.
2. Di Bisceglie AM. Hepatic tumors. In: Lee Goldman, Andrew I. Schafer, editors. Cecil Textbook of Medicine. 2nd Ed. Philadelphia, Pennsylvania, USA, Elsevier; c2004. p. 1222-6.
3. Fallon MB. Hepatic tumors. In: Lee Goldman, Andrew I. Schafer, editors. Cecil Textbook of Medicine. 2nd ed. Philadelphia, Pennsylvania, USA, Elsevier; c2004. p. 1222-6.
4. Bosch FX, Ribes J, Díaz M, Cléries R. Primary liver cancer: Worldwide incidence and trends. Gastroenterology. 2004;127:16-22.
5. Watany M, Badawi R, Elkhalawany W, Abd-Elsalam S. Study of *Dickkopf-1* (*DKK-1*) Gene Expression in Hepatocellular Carcinoma Patients. J Clin Diagn Res. 2017;11:32-4.
6. Zeeneldin AA, Salem SE, Darwish AD, El-Gammal MM, Hussein MM, Saadeldin M. Untreated hepatocellular carcinoma in Egypt: Outcome and prognostic factors. J Hepatocell Carcinoma. 2015;2:3-9.
7. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
8. Petrick JL, Florio AA, Znaor A, Ruggieri D, Laversanne M, Alvarez CS, *et al*. International trends in hepatocellular carcinoma incidence, 1978-2012. Int J Cancer. 2020;147:317-30.
9. Chiang CJ, Yang YW, You SL, Lai MS, Chen CJ. Thirty-Year Outcomes of the National Hepatitis B Immunization Program in Taiwan. JAMA. 2013;310:974-6.
10. Jinjuvadia R, Patel S, Liangpunsakul S. The association between metabolic syndrome and hepatocellular carcinoma: Systemic review and meta-analysis. J Clin Gastroenterol. 2014;48:172-7.
11. Villanueva A. Hepatocellular Carcinoma. N Engl J Med. 2019;380:1450-62.
12. Wang Y, Huang S, Zhang Y, Cheng Y, Dai L, Gao W, *et al*. Construction and validation of a prognostic model based on autophagy-related genes for hepatocellular carcinoma in the Asian population. BMC Genomics. 2023;24:357-65.
13. Sun S, Poon RT, Lee NP, Yeung C, Chan KL, Ng IO, *et al*. Proteomics of hepatocellular carcinoma: Serum vimentin as a surrogate marker for small tumors (≤ 2 cm). J Proteome Res. 2010;9:1923-30.
14. Hu L, Lau SH, Tzang CH, Wen JM, Wang W, Xie D, *et al*. Association of *Vimentin* overexpression and hepatocellular carcinoma metastasis. Oncogene. 2004;23:298-302.
15. Satelli A, Li S. *Vimentin* in cancer and its potential as a molecular target for cancer therapy. Cell Mol Life Sci. 2011;68:3033-46.
16. Kitamura Y, Shirahata A, Sakuraba K, Goto T, Mizukami H, Saito M, *et al*. Aberrant methylation of the *Vimentin* gene in hepatocellular carcinoma. Anticancer Res. 2011;31:1289-91.
17. Idriss NK, Fakhry M, Imam HM, Elmoez AFA, Abdelewahab H, Wahid AL, *et al*. Analysis of *Lamin B1*, *Vimentin* and *Anti-Ku86* as Prospective Biomarkers of Hepatocellular Carcinoma in Patients with Hepatitis C Virus Infection. Cell Physiol Biochem. 2019;52:595-605.

How to Cite This Article
Ramadan AM, Negm OA, Mohamed WS, Bendary AS. Study of vimentin serum level and gene methylation in patients with hepatocellular carcinoma. International Journal of Clinical and Diagnostic Pathology. 2024;7(3):24-29.