Al-Azhar Med. J . (Medicine) *Vol. 51 (1), January, 2022, 709-718*
DOI: 10.21608/amj.2022.212805
https://amj.journals.ekb.eg/article_212805.html

CHROMOGRANIN-A AS A NEW SERUM MARKER FOR DIAGNOSIS OF HEPATOCELLULAR CARCINOMA

By

Ahmed Abd El-Twab Abd El-Aziz[1]*, Ibrahim M. El-hosiny[1], Heba Abdella[2] and Ayman Abd El-Aziz[3]

[1]Department of Microbiology, Faculty of Medicine, Al-Azhar University

[2]Tropical Medicine Department, Faculty of Medicine, Ain Shams University

[3]Theodore Bilharz Research Institute

***Corresponding Author:** Ahmed Abd El-Twab Abd El-Aziz

Mobile: (+02)01007549979**, E-mail:** ahmedabdeltawwab.206@azhar.edu.eg, mohanad_200941@yahoo.com, mohanad.200941@gmail.com

ABSTRACT

Background: Hepatocellular carcinoma (HCC) is the world's sixth most common cancer, and it usually complicates liver cirrhosis. Its prevalence is growing globally, with annual increases ranging from 3% to 9%. Despite the fact that the alpha fetoprotein (AFP) is the gold standard for HCC serodiagnosis, it has a high rate of false positive outcomes, which are associated with a bad diagnosis.

Objective: To investigate the potential role of chromogranin-A (CgA) as a diagnostic non-invasive marker for HCC and to assess its sensitivity and specificity in diagnosis of HCC compared to AFP.

Material and Methods: This study was conducted from October 2017 till March 2020 at the Department of Tropical Medicine, Ain Shams University and Theodor Bilharz research institute. The study included 35 patients diagnosed with hepatocellular carcinoma HCC and 35 patients with chronic liver diseases (CLD). The study also included 20 apparently healthy individual served as a healthy control group taken from the blood donors at the blood bank of Ain Shams University Hospital. AFP and CgA serum levels were detected for all groups using enzyme linked immunosorbent assay (ELISA).

Results: The results of the study showed that the median of the of CgA in the HCC group was 53.40 and in CLD group was 22.55 while in the control group was 15.30; which indicates a significant elevation of CgA in HCC patients.

Conclusion: CgA is one of the promising tumor markers in hepatic focal lesions for detection of HCC.

Keywords: Serum Chromogranin-A, Hepatocellular Carcinoma, Alpha-Fetoprotein.

INTRODUCTION

HCC is the sixth most common in the world, complicating liver cirrhosis *(Tae-Hyung et al., 2019)*. Its prevalence is growing globally, with annual increases ranging from 3% to 9%. HCC has been on the rise in Egypt, with the incidence rate doubling in the last decade, and this has been due to a number of causes including (e.g. viral hepatitis B, D and C virus infections), environmental influences (e.g. aflatoxin, AF of aspergillus) and other reasons such as cigarette smoking, occupational exposure to chemicals such

as pesticides, and endemic infections in the community, such as bilharziasis, may have further roles in the etiology or progress of this problem *(Mashaly et al.,2018).*

HCC is one of the major causes of death because of its high frequency and poor prognosis; it is responsible for about one million deaths annually with only few patients surviving beyond one year. It is one of the few human cancers for which an etiological factor can be identified in most cases.

HCC commonly develops in cirrhotic livers whatever the etiology, so that liver cirrhosis by itself represents the strongest risk factor *(Toyoda el al., 2015).*

Early detection of patients with HCC is very important because it gives better prognosis as HCC tends to grow slowly and stay confined to the liver. So early diagnosis of HCC is not difficult if tumor markers and medical imaging are combined *(Piñero et al., 2020* and *Toyoda et al., 2015).* HCC is a potential target for cancer surveillance as it occurs in well-defined, at-risk populations and curative therapy is possible only for small tumors. Hepatic ultrasonography with or without AFP performed every 6 months is the preferred program and has been well shown to detect small tumors for curative treatment, which may be translated to improved patient survival *(Biselli et al., 2015).* The only diagnostic serologic test currently used in clinical practice is AFP, but it has a high rate of false positives and its concentration may be elevated in some benign liver diseases, so new, more precise and sensitive markers are needed *(Piñero et al., 2020* and *Biselli et al., 2015).* Additionally, 20% to 30% of HCC patients had a negative AFP result, while some chronic hepatitis and cirrhosis patients had a positive AFP result. Furthermore, the AFP level in the blood does not always correlate to the clinical stage of HCC *(Wu et al., 2018).* CgA is an acid glycoprotein that was first discovered in the adrenal medulla's catecholamine storage vesicles. It is detected in low amounts in healthy people's blood, but elevated serum levels are a reliable predictor of carcinoid-like tumours and neuroendocrine tumours like neuroblastoma, pheochromocytoma, and small cell lung carcinoma *(Aluri et al., 2017).* Other factors, especially renal and cardiac function, can influence circulating CgA levels, and elevated serum CgA levels have been recorded in both kidney and heart failure *(Mikkelsen et al., 2017* and *Peng et al., 2016).*

Within HCC tissue, clusters of cells containing CgA have been discovered. Elevated serum CgA level was found in half of HCC patients indicates that CgA may be a useful HCC marker *(Gkolfinopoulos et al., 2017* and *Zhou et al., 2020).*

The present work was to evaluate the value of CgA as a marker for HCC.

PATIENTS AND METHODS

This study was conducted at Ain Shams University Hospital at the clinic of Hepatoma Group and Theodor Bilharz Research Institute for pick up patients with HCC and chronic liver disease CLD.

The studied groups were classified as follows:

1. The first group included thirty five patients already diagnosed with HCC by AFP and triphasic C.T.
2. The second group included thirty five patients already diagnosed with chronic liver diseases by liver function tests and abdominal ultrasound.
3. The third group was a control group containing twenty healthy individuals.

Exclusion criteria:

1. Patients with renal failure.
2. Patients with cardiac failure.
3. Patients with any malignancy elsewhere in the body, other than liver especially those with neuroendocrine origin.

Clinical assessment included full history, clinical examinations and Child's classification for HCC and CLD were done.

Laboratory investigations included liver function tests, renal function tests, AFP and CgA ELISA assays were done for all groups.

Radiological investigations included abdominal ultrasonography and triphasic abdominal CT were done for HCC and CLD groups.

Statistical analysis:

Statistical analysis was performed using statistical package for social science (SPSS) version 25. Chi-Square test X^2 and Fisher's Exact Test were used to test the difference in qualitative data. Receiver Operating Characteristic (ROC) curve was used to compare the significant results among the studied groups.

Qualitative data were presented as mean ± standard deviation (SD) for age and as median and inter-quantitative range for non-parametric values, which were compared by Kruskal-Wallis test and Bonferroni test as a post ROC test. All P-values represented were two-sided, and statistical significance was declared at $P< 0.05$.

RESULTS

There were no significant differences in demographic and clinical characteristics between the studied groups except for the lower limb edema which was increased among CLD group (<0.05). As regarding the ultra-sonographic findings, no statistically significant data were detected among the studied groups except for the presence of portal vein thrombosis among HCC group (<0.05).

A positive triphasic CT criteria was noticed among 29 patients of HCC group (82.9%) only.

There was a statistical significant difference between the three groups as regards the level of CgA, AFP, AST, bilirubin and prothrombin time ($P<0.05$). There was no statistical significant difference between the three groups as regards the ALT, albumin levels **(Table 1)**.

Table (1): Comparison between the three groups according to the level of CgA, AFP, AST, ALT, albumin, bilirubin and prothrombin time

Groups / Parameters	HCC			Chronic liver disease cases			control			P
	Median	IQR (25^{th}-75^{th})		Median	IQR (25^{th}-75^{th})		Median	IQR (25^{th}-75^{th})		
CgA	53.40	16.00	221.20	22.55	7.93	126.28	15.30	6.23	48.35	<0.05
AFP	40.00	12.90	592.00	4.37	2.00	12.30	3.10	.53	12.50	<0.001
AST	43.00	29.00	52.00	46.00	28.00	63.00	33.00	27.25	37.00	<0.05
ALT	42.00	27.00	64.00	43.00	28.00	59.00	33.50	27.00	40.50	>0.05
Albumin	3.40	2.80	3.90	3.80	3.10	4.10	3.85	3.53	4.10	>0.05
Bilirubin	1.20	.90	2.30	.80	.60	.90	.90	.80	.90	<0.001
Proth. time	15.00	13.65	16.05	13.80	13.00	14.80	15.45	14.93	16.00	<0.001

There was a statistical significant difference between the three groups as regards the distribution of +ve CgA, AFP **(Table 2)**.

Table (2): Comparison between the three groups according to CgA, AFP

Groups / Parameters		HCC		Chronic liver disease cases		control		P
		N	%	N	%	N	%	
α-FP	-ve	12	34.3%	34	97.1%	20	100.0%	<0.01
	+ve	23	65.7%	1	2.9%	0	.0%	
CgA	-ve	19	54.3%	26	74.3%	18	90.0%	<0.05
	+ve	16	45.7%	9	25.7%	2	10.0%	

AFP was normal in 12 cases of HCC and showed positive results in 23 cases, but CgA showed a positivity in 10 cases only out of 23 diagnosed by AFP and in 6 cases out of 12 noted as normal by AFP, when this 6 was added to 23 cases by AFP the results were 29+ve cases This combined use of CgA with AFP showed a sensitivity and specificity of 82% and 90%, respectively PPV 95% and NPV 75% with a diagnostic accuracy of 88% so, when AFP was normal, CgA serum values represented a complementary diagnostic tool **(Table 3)**.

Table (3): Total relation between AFP and CgA

AFP / HCC		Normal		+ve		Total	
		N	%	N	%	N	%
CgA	Normal	6	50.0%	13	56.5%	19	54.3%
	+ve	6	50.0%	10	43.5%	16	45.7%
Total		12	100.0%	23	100.0%	35	100.0%

On comparing HCC with CLD group:

AFP: showed a sensitivity and specificity of 66% and 97%, respectively, PPV 95% and NPV 74% with a diagnostic accuracy of 81%.

CgA: showed a sensitivity and specificity of 46% and 74%, respectively, PPV 64% and NPV 58% with a diagnostic accuracy of 60%.

The combined use of CgA with AFP did not improve the diagnostic indices of individual each marker it showed a sensitivity and specificity of 83% and 74%, respectively, PPV 76% and NPV 81% with a diagnostic accuracy of 79% **figures (1, 2)**.

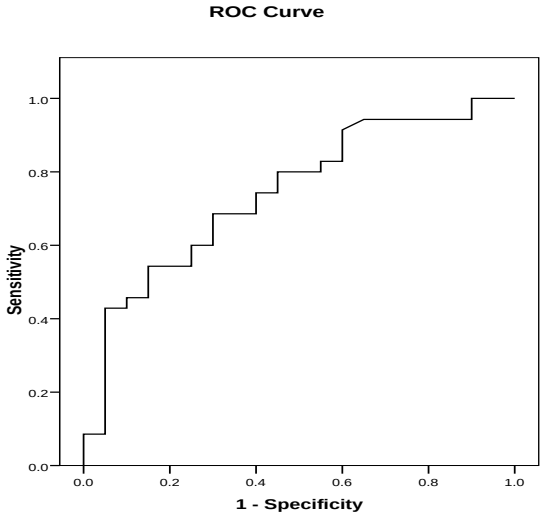

Figure (1): ROC curve for differentiation between normal and HCC is weak as AUC is 74%

*(AUC) Area under the curve.

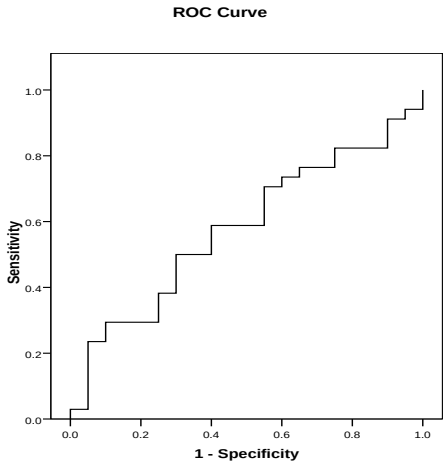

Figure (2): ROC curve for differentiation between CLD and HCC is weak as AUC is 66%

DISCUSSION

Since the hepatocarcinogenetic process can take years to progress from premalignant to overt HCC, surveillance of patients at risk allows for early detection of more treatable tumours *Yang et al. (2019)*. Ultrasonography (US) of the liver and serum AFP level, either separately or in combination, are the two most common tests used for screening *Zhou et al. (2020)*. The accuracy of ultrasonographic diagnosis is influenced by a number of factors, including the operator's skill, the sophistication of the equipment, and the size and nature of the tumor. Improvements in serological markers for screening patients at high risk of developing HCC could lead to earlier identification, intervention, and treatment success *Zhou et al. (2020)*. Alpha fetoprotein is the most well-established tumour marker in HCC, and it serves as the gold standard against which other markers are evaluated *Piñero et al. (2020)* and *Biselli et al. (2015)*. The normal range for serum AFP levels is up to 20ng/ml.

The presence of non-AFP-secreting tumors limits the sensitivity of AFP as a diagnostic method. As a result of the high number of false positive and false negative outcomes, AFP is no longer recommended for screening patients with CLD *Wang et al. (2020)*.

Patients with neuroendocrine tumours such as carcinoids and endocrine pancreatic tumours have chromogranin A (CgA) throughout their serum. CgA levels in the blood have also been shown to be high in patients with other cancers such as colon, lung, breast, and prostate cancer, likely due to a neuroendocrine distinction *Aluri and Dillon. (2017)*.

Although it is not clear why a non-neuroendocrine tumor such as HCC express CgA, it was noted that neuroendocrine differentiation can occur in carcinomas that lack neuroendocrine cells in their normal epithelial counterparts, such as hepatocellular carcinoma *(Aluri & Dillon. 2017*, and *Mikkelsen et al., 2017)*.

Interestingly, clusters of cells containing chromogranin A have been found within HCC tissue, and several studies have found elevated levels of serum CgA in HCC patients, implying that this marker may have a diagnostic role for HCC *(Berretta et al., 2017* and *Gkolfinopoulos et al., 2017)*.

Chromogranin A was abnormally elevated in 39 of the 79 patients with HCC examined by *Gkolfinopoulos et al., (2017)*, indicating a role for CgA in neuroendocrine differentiation of liver cells and the possibility of using it as a diagnostic marker for HCC.

The present study aimed at assessing the potential usefulness of CgA and its use as a marker in diagnosis of hepatocellular carcinoma.

In this study, there was a statistically significant elevation in the median serum AFP in HCC group (40ng/ml) when compared with control group (3.1 ng/ml) and significant elevation when compared with CLD group (4.37ng/ml). These findings were similar to many other studies but with different figures as *Awadallah et al. (2011)* who reported a significant elevation in the median serum level of AFP (26.5ng/ml) in HCC group when compared with the control group (2.75ng/ml) and significant elevation when compared with the CLD group

(5.35ng/ml). The same findings reported by *Biondi et al. (2012)* in the current study, the sensitivity of AFP was (66%) and the specificity was (97%). When using the receiver operator curve (ROC) to improve the specificity and sensitivity of AFP at a cutoff value of 20.4 ng/dl the detected sensitivity and specificity was 68.7% and 98.2%, respectively (best cut-off). These results were near to that detected by *Wu et al. (2019)*, who reported a sensitivity of 63% and specificity of 91% for AFP in diagnosis of HCC with a cut off value of 13.5 ng/dl.

In terms of using CgA as a marker for HCC diagnosis, our findings showed a statistically significant ($P<0.05$) increase in the median serum level of CgA in the HCC group (53.4ng/ml) when compared to the control group (15.3ng/ml) and a highly significant (22.55ng/ml) increase when compared to the cirrhotic group.

These findings were in line with those of *Awadallah et al. (2011)*, who found that the median serum level of CgA in the HCC community was (71.7 ng/ml) as opposed to the control group (15.8 ng/ml). In the studies of *Berretta et al. (2019)* and *Biondi et al. (2012)* the median serum CgA was (61.2 ng/ml and 60 ng/ml, respectively, in the HCC community.

However, our results did not match with the study of *Vezzosi et al. (2011)* who concluded that many false positive results of serum CgA were noticed among normal individuals or those with chronic diseases should be reevaluated by other techniques for accurate diagnosis of neuroendocrine tumors. The miserable results in their study may be attributed to the use of higher number of patients of HCC cases and inaccuracy of the method and techniques of CgA measurements.

Chromogranin-A's sensitivity and specificity were found to be 46% and 74%, respectively, in this study. At a cutoff value of 4.45 ng/dl, the yielded sensitivity and specificity were 49.7% and 80%, respectively, when using the ROC curve to increase the specificity and sensitivity of CgA (best cutoff). These findings were close to those of *Masayuki et al. (2014)* who registered a sensitivity of 53.7 percent and specificity of 79 percent with a cut off value of 6.75ng/ml and a sensitivity of 53.7 percent. Furthermore, *Masayuki et al. (2014)*, reported a higher sensitivity of CgA in patients with neuroendocrine tumors and can discriminate between neoplastic and non-malignant neuroendocrine disorders. Our findings are also consistent with those of *Biondi et al. (2012)* who found CgA to be highly important in the identification of HCC cases.

However, our findings were slightly differed from those of *Awadallah et al. (2011)* who registered a sensitivity of 83.3% and a specificity of 76.7% with a cut-off value of 28.78 ng/ml. Furthermore, our findings contradicted with those of *Masami et al. (2017)* who registered a sensitivity of 79% and specificity of 64% with a cut-off value of (19.5 ng/ml).

The combined use of CgA with AFP did not improve the net diagnostic results for detection of HCC cases. These findings were in line with those of *Biondi et al. (2012)* who concluded that CgA alone is a useful diagnostic marker for HCC and should be evaluated with or without AFP especially in chronic liver disease patients. This showed that

simultaneous measurements of serum AFP and CgA are of value in detecting HCC.

In the current study, there was no statistically significant difference between Barcelona HCC staging and CgA levels in the three groups. However, In a study performed by *Biondi et al. (2012),* CgA levels in the Barcelona staging of HCC were higher in stage D relative to stage C ($p<0.01$), stage B , and stage A.

According to *Lv et al. (2018),* the association between CgA and the degree of neuroendocrine inflammation indicates that CgA excretion could be involved during chronic inflammatory diseases as that in hepatitis and pancreatitis. These findings concluded that CgA levels are higher than usual in the early stages of chronic liver disease, which is why it should be tested. As a consequence, it should be viewed as a diagnostic marker for people with chronic liver diseases in order to diagnose early HCC and enhance their management outcomes.

CONCLUSION

CgA is one of the most promising tumour markers for detecting HCC in hepatic focal lesions. Chromogranin A serum levels can be used as a supplementary diagnostic instrument in the evaluation of chronic liver disease patients for the identification of HCC. It could also be used to diagnose HCC patients with focal lesions larger than 2 cm in diameter, especially when AFP levels were ambiguous despite the presence of a hepatic focal lesion on abdominal ultrasound and triphasic CT.

REFERENCES

1. **Aluri V and Dillon JS (2017):** Biochemical testing in neuroendocrine tumors. Endocrinology and Metabolism Clinics., 46(3): 669-77.
2. **Awadallah AM, Issa HA and Soliman MS (2011):** Evaluation of serum chromogranin a as a useful tumor marker for diagnosis of hepatocellular carcinoma. J Am Sci., 7(1): 999-1007.
3. **Berretta M, Cavaliere C and Alessandrini (2017):** Serum and tissue markers in hepatocellular carcinoma and cholangiocarcinoma: clinical and prognostic implications. Oncotarget. 8(8): 14192.
4. **Biondi A, Malaguarnera G and Vacante M (2012):** Elevated serum levels of Chromogranin A in hepatocellular carcinoma. BMC Surgery. 12(1): 1-4.
5. **Biselli M, Conti F, and Gramenzi A (2015):** A new approach to the use of α-fetoprotein as surveillance test for hepatocellular carcinoma in patients with cirrhosis. British Journal of Cancer., 112(1): 69-76.
6. **Gkolfinopoulos S, Tsapakidis K and Papadimitriou K (2017):** Chromogranin A as a valid marker in oncology: Clinical application or false hopes?. World Journal of Methodology. 7(1): 9-15.
7. **Hijioka M, Ito T, Igarashi H, Fujimori N, Lee L, Nakamura T, Jensen RT, Takayanagi R (2014):** Serum chromogranin A is a useful marker for Japanese patients with pancreatic neuroendocrine tumors. J Cancer Sci.; 105(11):1464-71.
8. **Lv Y, Han X, Zhang C, Fang Y, Pu N, Ji Y, Wang D, Xuefeng X and Lou W (2018):** Combined test of serum CgA and NSE improved the power of prognosis prediction of NF-pNETs.Endocr Connect., 7(1):169-178.
9. **Malaguarnera M, Vacante M, Fichera R, Cappellani A, Cristaldi E and Motta M (2010):** Chromogranin A (CgA) serum level as a marker of progression in hepatocellular carcinoma (HCC) of elderly patients. J Arch Gerontol Geriatr. 51(1):81-5.
10. **Mashaly AH, Anwar R and Ebrahim MA. (2018):** Diagnostic and prognostic value of talin-1 and midkine as tumor markers in hepatocellular carcinoma in Egyptian patients.

Asian Pacific journal of cancer prevention: APJCP; 19(6): 1503.

11. **Miki M, Ito T, Hijioka M, Lee L, Yasunaga K, Ueda K, Fujiyama T, Tachibana Y, Kawabe K, Jensen RT, Ogawa Y (2017):** Utility of chromogranin B compared with chromogranin A as a biomarker in Japanese patients with pancreatic neuroendocrine tumors. Jpn J Clin Oncol., 1; 47(6):520-528.
12. **Mikkelsen G, Åsberg A and Hultström ME (2017):** Reference limits for chromogranin A, CYFRA 21-1, CA 125, CA 19-9 and carcinoembryonic antigen in patients with chronic kidney disease. The International Journal of Biological Markers, 32(4): 461-6.
13. **Peng F, Chu S and Ding W (2016):** The predictive value of plasma catestatin for all-cause and cardiac deaths in chronic heart failure patients. Peptides, 86: 112-7.
14. **Piñero F, Dirchwolf M and Pessôa MG (2020):** Biomarkers in hepatocellular carcinoma: Diagnosis, prognosis and treatment response assessment. Cells. 9(6): 1370.
15. **Tae-Hyung Kim, So Yeon Kim, An Tang and Jeong Min Lee (2019):** Comparison of international guidelines for noninvasive diagnosis of hepatocellular carcinoma. Clin Mol Hepatol. 25(3): 245–263.
16. **Toyoda H, Kumada T and Tada T (2015):** Tumor markers for hepatocellular carcinoma: simple and significant predictors of outcome in patients with HCC. Liver Cancer, 4(2): 126-36.
17. **Vezzosi D, Walter T, Laplanche A, Raoul JL, Dromain C, Ruszniewski P, d'Herbomez M, Guigay J, Mitry E, Cadiot G, Leboulleux S, Lombard-Bohas C, Borson-Chazot F, Ducreux M and Baudin E (2011):** Chromogranin A measurement in metastatic well-differentiated gastro-entero-pancreatic neuroendocrine carcinoma: screening for false positives and a prospective follow-up study. Int J Biol Markers, 26(2):94-101.
18. **Wang T and Zhang KH (2020):** New Blood Biomarkers for the Diagnosis of AFP-negative Hepatocellular Carcinoma. Frontiers in Oncology, 10: 1316.
19. **Wu G, Wu J, Pan X, Liu B, Yao Z, Guo Y, Shi X and Ding Y (2019):** Racial disparities in alpha-fetoprotein testing and alpha-fetoprotein status associated with the diagnosis and outcome of hepatocellular carcinoma patients. J Cancer Med, 8(15):6614-6623.
20. **Wu M, Liu H and Liu Z (2018):** Analysis of serum alpha-fetoprotein (AFP) and AFP-L3 levels by protein microarray. Journal of International Medical Research, 46(10): 4297-305.
21. **Yang JD, Hainaut P and Gores GJ (2019):** A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nature reviews Gastroenterology & Hepatology, 16(10): 589-604.
22. **Zhou J, Sun H and Wang Z (2020):** Guidelines for the Diagnosis and Treatment of Hepatocellular Carcinoma (2019 Edition). Liver Cancer. 9(6): 682-720.

كروموجرانين- أ كدلالة لتشخيص سرطان الكبد

احمد عبد التواب عبد العزيز*، ابراهيم محمد الحسينى*، هبة عبد اللاة، ايمن عبد العزيز*****

***قسم الميكروبيولوجى، طب الازهر بالقاهرة**

****قسم الامراض المتوطنة طب عين شمس**

*****معهد تيودوربلهارس**

E-mail: ahmedabdeltawwab.206@azhar.edu.eg

خلفيةالبحث: يعتبر سرطان الكبد من اكثر الاورام شيوعا حول العالم وعلى الرغم من الاعتماد على الالفا فيتوبروتين كدلالة غيرغازية لتشخيصة الا انة يحمل نسب خطأ كثيرة فى اكتشاف الورم وعلية تصبح الحاجة داعية لوجود دلالات اخرى للتشخيص.

الهدف من البحث: تقييم كروموجرانين- أ كدلالة غيرغازية لتشخيص سرطان الكبد.

المرضى وطرق البحث: شمل البحث ثلاث مجموعات الاولى تمثل حالات سرطان الكبد وشملت 35 حالة والثانية مجموعة التهاب الكبد المزمن وشملت 35 حالة والثالثة مجموعة ضابطة وشملت 20 حالة. تم الكشف عن الالفا فيتو بروتين و كروموجرانين- أباستخدام الاليزا وذلك فى الثلاث مجموعات والمقارنة بينهم.

نتتائج البحث: تبين ارتفاع ملحوظ فى نسبة كروموجرانين-أ بين حالات المجموعة الاولى والتى مثلت حالات سرطان الكبد.

الاستنتاج: كروموجرانين- أ واحد من الدلات الواعدة الغير غازية والتى يمكن الاعتماد عليها فى تشخيص سرطان وبؤر الكبد الخبيثة.

الكلمات الدالة: كروموجرانين- أ بالمصل، سرطان الكبد، الفافيتوبروتين.