COMPARISON BETWEEN HEPATOCELLULAR CARCINOMA &COLORECTAL CANCER BY USING SOME TUMOR MARKER[+]

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Abstract:

In this study 40 Iraqi patients complaining from organs cancer were studied, they were categorized into: two groups (liver, colon), each group comprises 20 patients diagnosed by histopathological examination.A sample of 20 healthy persons was studied as a control group.

The incidence of cancer was higher in patients at age (30-49) years in comparison with other age range group.

The results showed that the incidences of Hepatocellular carcinoma and colonic carcinoma (HCC) in male patients were higher when compared with female patients afflicted with the same organ disease .

The study illustrated that there was a highly significant increase in mean concentration . of AFP (51 ng/ml) in serum of patients afflicted with HCC when compared with other organs cancer and control.

The results showed that the mean concentration. of carcino embryonic antigen (CEA) was higher in patients with colon cancer (26.3 ng/ml) when compared with liver cancer and control group.

المستخلص :

شمل هذا البحث دراسة ٤٠ مريضا ومريضة يشكون من مرض السرطان في ألاعضاء وتم تصنيفهم إلى مجموعتين: (مرضى سرطان الكبد، وسرطان القولون) كل مجموعة تتكون من ٢٠ مريضاً حيث تم التشخيص اعتمادا على الفحص النسيجي كما تم اختيار (٢٠) شخصا من الأصحاء باعتبارهم مجموعة سيطرة.

أظهرت النتائج وجود علاقة واضحة مابين العمر ونسبة الإصابة بالسرطان للأعضاء المختلفة حيث آن الفئة العمرية ما بين (٣٠-٤٩سنة) هي أكثر فئة إصابة بالسرطان مقارنة مع الفئات العمرية الأخرى المصابة بالسرطان.

أوضحت الدراسة آن حدوث الإصابة بسرطان الكبد والقولون في الذكور أعلى منه من الإناث وبفرق معنوي ($P<0.05$).

أظهرت الدراسة وجود فرق عالي المعنوية في معدل تركيز البروتين الجنيني الألفي (α-Fetoprotein) (٥١ نانو غرام/مل) في مصل المرضى المصابين بسرطان الكبد الخبيث مقارنة مع مرضى سرطان القولون والسيطرة.

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أشارت الدراسة عدم وجود فرق معنوي في معدل تركيز البروتين الجنيني الألفي في مرضى القولون (P<0.05).

اظهرت النتائج وجود زيادة في معدل تركيز المستضد البروتيني السرطاني (CEA) في مصل المرضى المصابين بسرطان القولون اذ كان (٢٦,٣ نانوغرام/ مل) مقارنة مع مرضى سرطان الكبد والأشخاص الأصحاء (٣,٩،٨) على التوالي وبفرق معنوي (P< 0.01).

تبين من خلال الدراسة عدم وجود فرق معنوي في معدل تركيز البروتين الجنيني المستضد في مصل المرضى المصابين بسرطان الكبد P< 0.05).

Introduction:

- Fetoprotein is a glycoprotein (4% carbohydrate) with molecular size of approximately 70,000 Dalton and contains an asparagin binding, double-chain complex sugar, with $\alpha 1$ electrophoretic mobility [1].

It exists in sera of healthy individuals in concentrations around 10 ng/ml, twice the upper limit of normal (20 ng/ml) is used as the cutoff value for diagnosing HCC.[2].

Alpha-Fetoprotein is a normal fetal serum protein synthesized by the liver, Yolk Sac, and gastrointestinal tract that shares sequence homology with albumin . It is a major component of fetal plasma, reaching a peak concentration of 3 ng/ml at 12 weeks of gestation, following birth, it clears rapidly from the circulation, having a half life of 3.5 days, and it concentration in adult serum is less than 20 mg/ml.[3].

The expression of AFP gene has been looked into, which should be reflected by increased levels of transcribed mRNA levels. The level of AFP mRNA in peripheral nucleated blood cells are reported to serve as a marker for micrometastases of Hepatocellular carcinoma and colonic carcinoma (HCC) , but they have not been widely used in clinical practice so far.[4].

In the embryo, transcription from the AFP gene occurs in the liver and yolk sac, early in development transcription from the albumin gene begins in fetal hepatocyte while transcription from AFP gene continuous. After birth, transcription from AFP gene greatly decrease and transcription of the Albumin continuous. The AFP gene remains essentially transcriptionally silent in hepatocytes during adulthood unless reactivated in HCC or regenerally cells.[5].

Recent prospective trail from France demonstrated 78% sensitivity and 93% specificity for the detection of HCC in-patients with established cirrhosis[6].

carcino embryonic antigen (CEA) is found on the surface membrane of normal and malignant cells. Monoclonal antibodies have been prepared to at least six distinct epitopes on CEA.[7].

Circulating CEA is found in different stage and increased with advance stages of tumors [8].

CEA is also expressed in other carcinoma such as gastric, lung cancer but is lower significantly than in colorectal cancer [9].

Materials & Methods:

Various blood samples were collected from 40 patients with different organ malignancy and 20 samples were taken from control group, by means of disposable syringe. Blood was centrifuged for obtaining serum for measurement of tumor markers (CEA and AFP).

All samples were taken from patients attending to gastroentrology and hepatology center in Baghdad city, for the period from October 2005 to April 2006, to estimate AFP&CEA by using enzyme immunoassay test . All procedures were achieved by following the manufacture's directions of Biocheck company.

Estimation of carcinoembryonic antisen (CEA) enzyme immunoassay test.

- 50 µL of standard, specimens and control was dispensed into appropriate wells.
- 100 µL of enzyme conjugate was dispensed into each well.
- Mixing thoroughly for 60 seconds.
- The plate was incubated at room temperature (18-25°C) for 60 minutes.
- The incubation mixture was removed by employing plate content into a waste container.
- The microtiter plate wells were rinsed 5 times with distilled water.
- Residual water droplets were striked the wells onto absorbent paper.
- 100 µL of TMB reagent was dispensed into each well with gently mixing for 5 seconds.
- Re-incubation at room temperature for 20 minutes.
- - The reaction was stoped by adding 100 µL of stop solution to each well with gently mixing for 30 seconds.
- - The optical density 450 nm with a microtiter reader was read with 15min.
- Then standard curve was plotted to determine the concentration of CEA in patient serum (according to protocol of Biocheck , Inc)

Estimation of AIpha-Fetoprotein enzyme immunoassay test:

- - 20µL of standard, specimens and control was dispensed into appropriate wells.
- - 100 µL of zero Buffers was dispensed into each well.
- - Mixing thoroughly for 30 seconds.
- - The plate was incubated at room temperature (19-25°C) for 30 minuets.
- - The incubated mixture was removed by flicking plate content into a waste container.
- - The microtitor plate wells were rinsed 5 times with distilled water.
- - Residual water droplets were striked the wells onto absorbent paper. Then 150uL of enzyme
- conjugate agent was dispensed into each well with gently mixing for 5 seconds.
- - The plate was re-incubated at room temperature for 30 minutes.
- - The incubated mixture was removed by flicking plate contents into waste content.
- - The microtiter wells were rinsed and flicked 5 times with distilled water.
- - Residual water droplets were striked onto absorbent paper.

- - 10 µL of TMB reagent was dispensed into each well with gently mixing for 5 seconds.
- - Re-incubation at room temperature for 20 minutes.
- - The reaction was stopped by adding 100 µL of stop solution to each well & with gently
- mixing for 30 seconds.
- - The optional density 450 nm with amicrotiter reader was read with 15 min.

Then standard curve was plotted to determine the concentration of CEA in patient serum (according to protocol of Biocheck , Inc)

Results:

Table (1): Distribution of patients with cancer according to age range group.

Age group by years	*Organs*	*No.*	*%*	*C.S. by Ks-test*
10 – 29 *(1)*	Liver	3	15	NS (0.250) P > 0.20
	Colon	5	25	
30 – 49 (2)	Liver	9	45	NS (0.200) P > 0.20
	Colon	5	25	
50 – 69 (3)	Liver	5	25	HS (0.167) P> 0.20
	Colon	8	40	
70 – 89 (4)	Liver	3	15	NS (0.150) P> 0.20
	Colon	2	10	

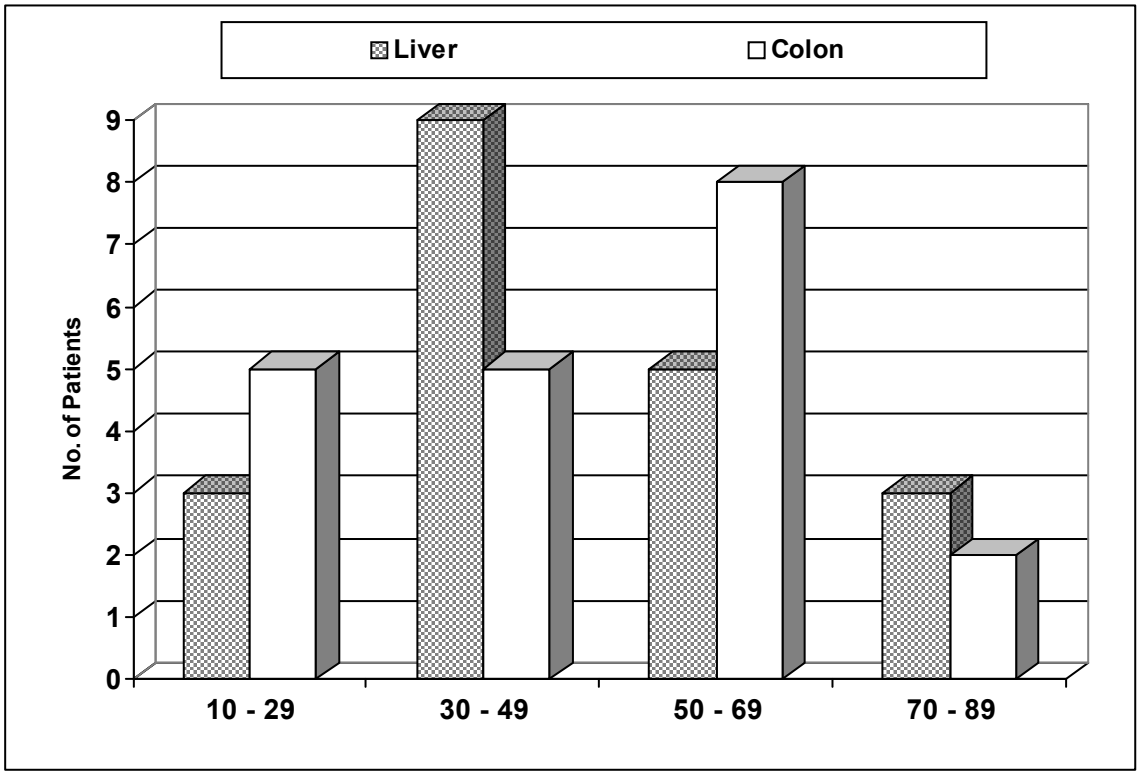

Figure (1): Distribution of patients with cancer according to age range group

Table (2): Distribution of patients with cancer according to Sex.

Organ	*Sex*	*No.*	*%*	*C.S. by z-test*
Liver	***Male***	13	65	**S P = 0.041**
	Female	7	35	
Colon	***Male***	12	60	**S P = 0.04**
	Female	8	40	

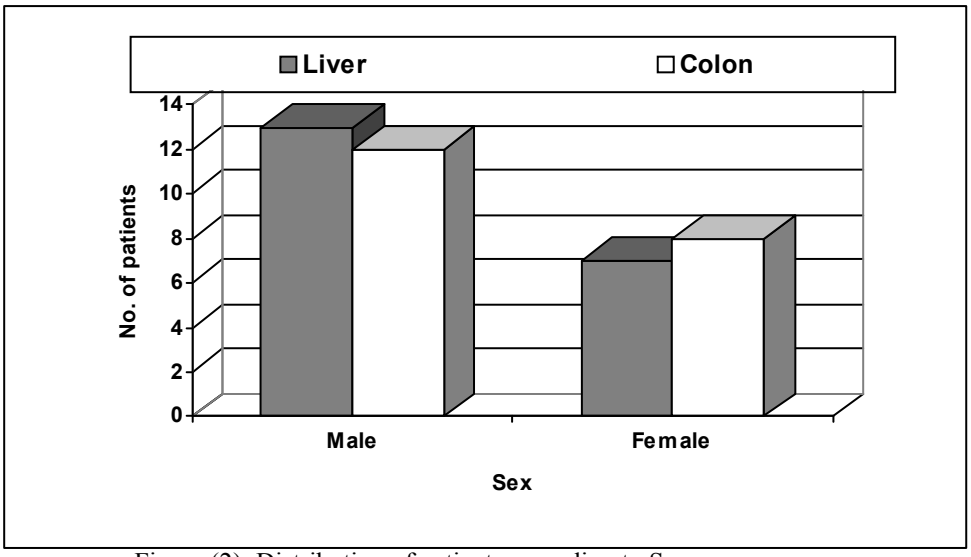

Figure (2): Distribution of patients according to Sex**.**

Table (3): Mean concentration of α-Fetoprotein (ng/ml) of patients with different organs cancer

Organ	*Mean conc. Of α-fetopein ng/ml*	*Std/ deviation*
Liver	51.0	9.435
Colon	19.3	3.201
Control	4.9	1.868

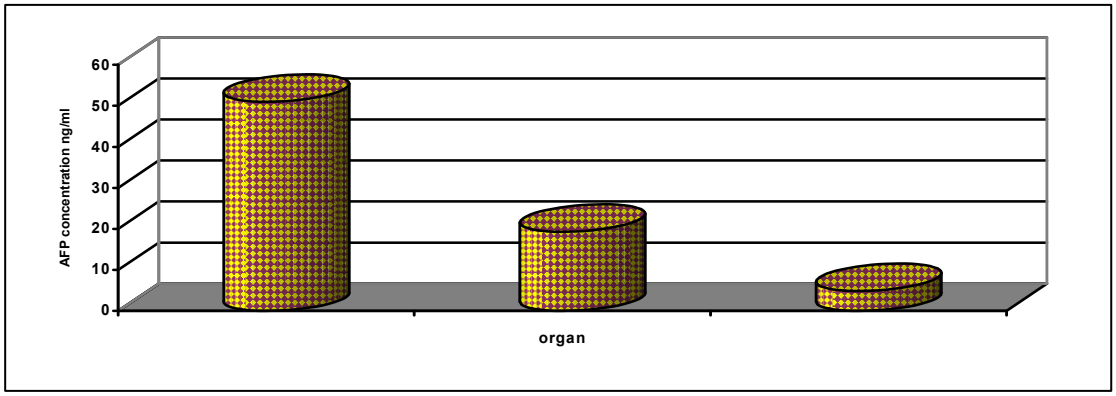

Figure (3): Mean concentration of α-Fetoprotein (ng/ml) of patients with different organs cancer

Table (4): Mean concentration of CEA(ng/ml) of patients with different organs cancer

Organ	*Mean conc. of carcinoembryoic antigen ng/ml*	*Std/ deviation (±)*
Liver	**8.0**	**3.768**
Colon	**26.3**	**7.649**
Control	**3.9**	**1.076**

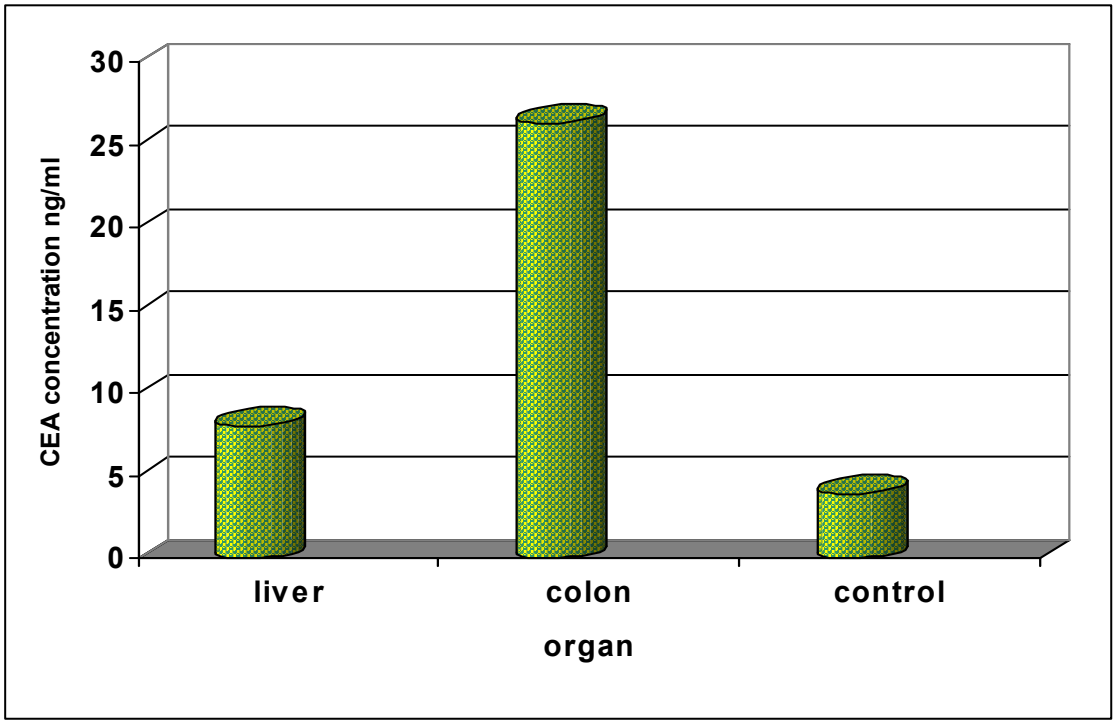

Figure (4): Mean concentration of CEA(ng/ml) of patients with different organs cancer

Table (5): Mean concentration of AFP and CEA in different organs cancer.

rgan	*Mean conc. of α-fetopein ng/ml*	*Mean conc. of CEA ng/ml.*	*C.S.*

Liver	**51.0**	**8**	**H.S P<0.01)**
Colon	**9.3**	**26.3**	**H.S (P<0.01)**

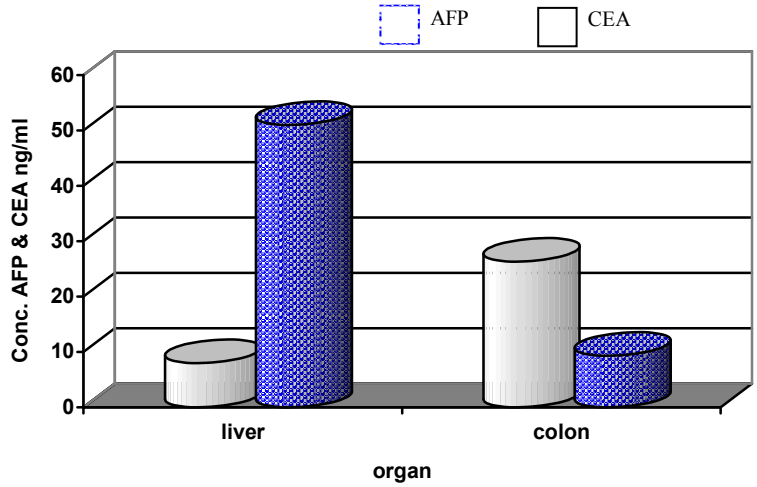

figure (5): Mean concentration of AFP and CEA in different organs cancer.

Discussion & Conclusions:

Table and Figure (1) showed that there were a high incidence of disease in patients with age range group (30-49) with different organ tumor studied when compared with other age range groups (P<0.01). The same table and figure showed that there were no significant difference between different organs cancer studied at the same age range group (30-49) (P>0.05).Our results agreed with many result obtained by many scientists, cancer was higher in at age (50-59) years old.[10].

The Data illustrated in table and figure (2) showed that the number and percentage of male patients afflicted with liver and colon were higher when compared with female patients afflicted with the same organ disease with significant difference (P<0.05).Our data agreed with result obtained by Tsuruta who said that the incidence of liver cancer was higher in male than female, due to contact of male with many carcinogenic substances as a result of job position [11] .

The data represented in table and figure (3) showed that there was a highly significant increase in mean concentration of α-fetoprotein (51.0 ng/ml) in serum of patients afflicted with liver cancer when compared with colon and control group (19.3 and 4.9 ng/ml) respectively (P<0.01).

The same table and figure showed that there were a highly significant increase in mean concentration of α-fetoprotein in patients with liver cancer when compared with colon and control group (P<0.01), While there was no significant difference in mean concentration of α-fetoprotein in patients with colon (P>0.05).

The results plotted in table and figure (4) showed that the mean concentration of CEA was higher in patients with colon cancer (26.3 ng/ml) when compared with liver and control group (8.0 and 3.9 ng/ml) respectively with highly significant (P<0.01).

The same table & figure showed that there was a highly significant increase in mean concentration of CEA in patients with colon cancer when compared with liver and control group (P<0.01), While there was no significant difference in mean concentration of CEA in patients with liver (P>0.05).

The results of this study agreed with many studies. One published report which stated that AFP is frequently used as a diagnostic marker for HCC. Serum AFP elevation has been known to be associated with HCC [12].

The second study stated that 90% of patients with HCC had elevated serum AFP levels above 100ng/ml [13].

The third study indicated that HCC was first diagnosed by elevated serum AFP values (<50ng/ml).

The mean concerntaion of serum AFP was (37±10 ng/ml) in tumor (1 cm diameter), 41±8 ng/ml in tumor (1.1 to 2 cm) and 36 ±12 ng/ml in tumor (2.1-3 cm) respectively [14].

The Fourth study reported that the low incidence of elevation of AFP occur in variety of tumors, especially colon.

The measurement of serum AFP is important for diagnosis for HCC. AFP is not screening but a diagnosis test [15].

The mean finding of this study was that patients with colon cancer have a higher level of serum CEA measured by enzyme linked immuno sorbebent assay (ElISA) technique and the value given by ng/ml in patients with colon cancer. Similar results were observed by many studies done all over the world.

One study showed that the mean value of preoperative serum CEA was more than twenty (20 ng/ml) [16].

A Second study stated that serum CEA level remains the best marker for the early diagnosis in patients with colorectal cancer, CEA and C19-9 showed increased levels in patients with colorectal cancer, CEA Conc. was first increased at early diagnosis [17].

Another study indicated that CEA marker was the most sensitive marker in diagnosis of early stage of colorectal cancer with C19-9 the least sensitive marker and hence showed not to be used for the study of this kind of study.

CEA remains the most reliable marker for the follow-up of colon cancer patients. The forth study observed that CEA marker is the most useful tool for the early detection of liver metastasis in patients with diagnosed colorectal cancer[18].

The fifth study showed that CEA has been widely accepted as a tumor marker useful in the diagnosis and management of colorectal carcinoma [19].

Table and figure (5) showed that the mean concentrations of AFP was higher in patients afflicted with liver cancer (51ng/ml) when compared with serum conc. of CEA (8 ng/ml) in the same patients with highly significant difference ($P<0.01$), while the mean concentration of CEA in patients with colon cancer was higher (26.3ng/ml) when compared with mean concentrations . Of AFP (9.3ng/ml) of the same patients.

Our data was agreed with the data obtained by Toriliz and webb whom said that the serum conc. of AFP is a diagnostic tool for diagnosis of patients with HCC, while serum concentrations of CEA is a considered as tool for diagnosis of patients with colon cancer [20].

There was a significant increase in the mean concentration of AFP in patients with HCC , the mean con. of CEA was higher in patients with colon cancer ,AFP is considered as efficient diagnostic tool for diagnosis and follow up of patients with HCC while CEA marker considered as powerful marker for diagnosis and follow up of patients with colon cancer.

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