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**INTERNATIONAL JOURNAL OF
 ADVANCED RESEARCH (IJAR)**

Article DOI: 10.21474/IJAR01/5954
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/5954>



RESEARCH ARTICLE

SERUM GOLGI PROTEIN 73 IN PATIENTS WITH HEPATOCELLULAR CARCINOMA.

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Manuscript Info

Manuscript History

Received: 03 October 2017
 Final Accepted: 05 November 2017
 Published: December 2017

Key words:-

Golgi Protein 73, Hepatocellular Carcinoma.

Abstract

Background: Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide. The diagnosis of HCC is mainly based on a combination of abdominal ultrasound and serum alpha fetoprotein (AFP) level. Serum AFP has low sensitivity, serum Golgi protein 73 (sGP73) is a novel and promising biomarker for detection of hepatocellular carcinoma (HCC). However, there are few reports on the predictive values levels of GP73 in diagnosis of HCC. We aimed to evaluate the clinical usefulness of serum GP73 in the diagnosis of HCC. **Methods:** The study was conducted on 80 patients included 20 patients with chronic hepatitis C (group I), 20 patients with liver cirrhosis (group II divided into two subgroups of equal number compensated and decompensated liver cirrhosis), 40 patients with HCC (group III) in addition to 10 apparently healthy control subjects (group IV). All patients in this study were subjected to full history taking, thorough clinical examination, radiological investigations, routine laboratory investigations and serum AFP in addition to serum GP73 assay by ELISA technique. **Results:** Assessment of the diagnostic performance of serum GP73 and serum AFP in the present study in diagnosis of HCC revealed that serum GP73 at cutoff of 11.1 ng/mL showed a diagnostic sensitivity of 82.5%, specificity 70%, positive predictive value 78.6%, negative predictive value 75% and the area under the curve (AUC) was 0.8025, Regarding serum AFP at cutoff 320ng/mL showed the diagnostic sensitivity of 79.41%, specificity 60 %, PPV 65.9%, NPV 75 %, AUC 0.766, This proved the superiority of GP73 estimation over AFP assay in cases of HCC. **Conclusion:** Serum GP73 had an overall performance better than AFP in detection of HCC in patients with chronic HCV related liver disease so it can be used in diagnosis of HCC.

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Introduction:-

Hepatocellular carcinoma (HCC) is one of the most common, aggressive solid malignancies worldwide, accounting for more than two-thirds of all primary liver cancer cases (Yang et al, 2015).

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Every 30 seconds, one person in the world dies from liver cancer, which is almost entirely preventable. The annual global death rate from HCC of just under 700,000 approximates the annual incidence, reflecting the limited therapeutic options as well as the late diagnosis in most cases (Marrero, 2013).

The most common risk factors of HCC are chronic hepatitis and liver cirrhosis caused by HBV and HCV infection (Braicu et al., 2009).

Cirrhotic and dysplastic nodules can mimic HCC and hence diagnosis of small tumors based on imaging is relatively inaccurate so this technique cannot differentiate benign hepatic lesions from HCC (Saar and Kellner, 2008), histopathological examination of tumor biopsy is considered the crucial method for reliable diagnosis of HCC, ultrasound-guided fine needle biopsy accurately diagnoses HCC in about 90% of nodules, including nodules of a very small diameter. However, malignant seedling is a recognized complication in patient with HCC and risk of tumor seedling along the needle tract has been estimated as 3% of cases (Change et al., 2008 and Gomaa et al., 2009).

Potentially curative treatment (surgery) is limited and really possible only for cases with small HCC malignancies, for this reason more effective surveillance strategies should be used to screen for early occurrence of HCC targeted to the population at risk. HCC diagnosis is a multistage process including clinical, laboratory, imaging and pathological examinations. So far, the generally accepted serological marker is α -fetoprotein (AFP), its diagnostic accuracy is unsatisfactory and questionable because of low sensitivity, therefore there is a strong demand by clinicians for new HCC-specific biomarkers (Stefaniuk et al., 2010).

Golgi protein 73 (GP73) also named Golgi phosphoprotein 2 (GOLPH2) is 73 kDa transmembrane glycoprotein that normally resides within the cis-Golgi complex (Marrero et al., 2005).

Some studies demonstrated significant elevation of serum level of GP73 in diverse viral and non-viral liver diseases, including hepatitis and cirrhosis (Fimmel and Wright, 2009). Moreover, up-regulated GP73 was observed in several malignances such as prostate cancer, and renal cell cancer. However, knowledge concerning GP73 function and the mechanisms of regulation in normal and neoplastic tissues is still under research (Zhou et al., 2011).

Patients and Methods:-

This study was conducted at the Department of Hepatology, Gastroenterology and Infectious Diseases at Benha University Hospital on 80 patients in addition to 10 persons of apparently healthy individuals with normal routine laboratory investigations and negative for both HCV Ab and HBsAg served as a control group, during the period from January 2016 to September 2016 after approval of Benha university ethical committee. Subjects included in this study were classified into the following groups: **Group I** : included 20 patients with chronic hepatitis C, **Group II**: included 20 patients with liver cirrhosis divided into two subgroups of equal number (compensated liver cirrhosis and decompensated liver cirrhosis), Liver cirrhosis is diagnosed by clinical, laboratory and U/S assessment, **Group III**: included 40 patients with hepatocellular carcinoma diagnosed by ultrasonography (U/S) and confirmed by triphasic computed tomography and **Group IV**: included 10 apparently healthy subjects served as control group.

All the patients and controls were subjected to full history taking, complete clinical examination, complete liver function tests, serum alpha fetoprotein (AFP). Abdominal ultrasonography was done and triphasic computed tomography when there was a lesion suspected of HCC on ultrasound. Serum Golgi Protein 73 concentration was quantitatively measured using an Enzyme-Linked Immuno-Sorbant Assay (ELISA) kit (sun red biotechnology company) according to manufacturers' instructions.

Statistical Analysis:-

The collected data were summarized in terms of mean \pm Standard Deviation (SD) and range for quantitative data and frequency and percentage for qualitative data. Comparisons between the different study groups were carried out using the Chi-square test (χ^2) and Fisher Exact test (FET) to compare proportions as appropriate.

The Mann-Whitney test (z) was used to compare two non-parametric data. One-way Analysis Of Variance (ANOVA; F) and Kruskal Wallis (χ^2) test were used to compare more than two groups regarding parametric and non-parametric data respectively. Spearman Correlation coefficient (rho; ρ) was used to test for the correlation of between estimated parameters. Receiver Operating Characteristic (ROC) analysis was carried out to evaluate the

diagnostic performance of plasma AFP and serum Golgi protein 73 for HCC screening among patients with chronic liver diseases. The best cutoff point and the corresponding sensitivity and specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Area Under the Curve (AUC) were estimated. After the calculation of each of the test statistics, the corresponding distribution tables were consulted to get the “P” (probability value), Statistical significance was accepted at P value <0.05 (S) while a P value >0.05 was considered non-significant. The statistical analysis was conducted using STATA/SE version 11.2 for Windows (STATA corporation ,College Station, Texas).

Results:-

Clinical history of all studied groups was shown in (Table 1). The comparison between HCC, liver cirrhosis, chronic hepatitis C and controls revealed that there were significant increase in AST, ALT, total bilirubin , INR in hepatitis C, cirrhosis (decompensated and compensated) and HCC groups compared to control group with the highest level of total bilirubin and INR in HCC group , highest level of AST and ALT in chronic hepatitis C group. In contrast, serum albumin level, hemoglobin concentration and platelet count were significantly decreased in HCC, cirrhosis and chronic hepatitis C groups as compared to controls (Table 2).

The serum level of GP73 was significantly more elevated in HCC group as compared to other groups as shown in (Table 3).

A significant positive correlation were found between serum GP73 and (AST , ALT, total bilirubin , INR and AFP) ,a significant negative correlation were found between serum GP73 and (platelet count and serum Albumin) , while there were no correlation between serum GP73 and (hemoglobin and TLC) as shown in (Table 4).

As regards tumor characters, there was a significant positive correlation between serum GP73 with number of focal lesions, there was no correlation between serum GP 73 and size of focal lesions as shown in (Table 5).

As regards prognostic markers of HCC, there were a significant positive correlation between serum GP73 with (Okuda staging and Child classification) as shown in (Table 6).

Regarding serum GP73 (ng/mL), at a cutoff of 11.1 ng/ mL showed a diagnostic sensitivity of 82.5%, specificity 70%, positive predictive value 78.6%, negative predictive value 75% .The area under the curve (AUC) was 0.8025 as shown in (Figure 1). As regard serum AFP, the diagnostic performance at the cut off (320ng/mL), showed the diagnostic sensitivity of 79.41%, specificity 60 %, PPV 65.9% , NPV 75 %, AUC 0.766 as shown in (figure 2). However when GP73 used in combination with AFP showed sensitivity of 82.5%, specificity 80 %, PPV 84.62%, NPV 77.42%, AUC 0.8842 as shown in (figure 3).

Table (1):- Clinical History in all studied groups

Variable	Group I (Chronic HCV) (no.=20)		Group II a (Compensated Liver Cirrhosis) (no.=10)		Group II b (Decompensated Liver Cirrhosis) (no.=10)		Group III (HCC) (no.=40)		P*
	No.	%	No.	%	No.	%	No.	%	
Abd. pain	4	20	3	30	5	50	26	65	0.006 (S)
Jaundice	0	0	0	0	5	50	26	65	<0.001(S)
Upper GIT bleeding	0	0	0	0	4	40	25	62.5	<0.001(S)
Ascites	0	0	0	0	5	50	24	60	<0.001(S)
H. encephalopathy	0	0	0	0	4	40	12	30	0.002 (S)
Loss of weight	0	0	0	0	0	0	9	22.5	0.02 (S)

* Obtained using the Fisher Exact Test

P<0.05: significant difference

Table (2):- Laboratory findings in all studied groups.

Variable	Group I (Chronic HCV) (no.=20)	Group II a (Compensate d Liver Cirrhosis) (no.=10)	Group II b (Decompensate d Liver Cirrhosis) (no.=10)	Group III (HCC) (no.=40)	Group IV (control group) (no.=10)	Test	P
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD		
HB (gm/dl)	12.9 \pm 1.55	11.44 \pm 1.42	8.18 \pm 1.24	8.53 \pm 0.88	12.72 \pm 1.51	F=62.3 2	<0.00 1 (S)
Platelets x1000/cmm	300 \pm 70.82	190 \pm 64.64	107.8 \pm 46.32	108.12 \pm 43.57	312.9 \pm 81.1 5	F=48.6 2	<0.00 1 (S)
AST (aspartate aminotransferas e) (IU/L)	108.3 \pm 73.5 2	106.1 \pm 68.5	90.2 \pm 64.94	76.57 \pm 44.37	25.7 \pm 9.26	F= 4.34	0.003 (S)
ALT (alanine aminotransferas e) (IU/L)	85.45 \pm 50.0 2	75.1 \pm 46.35	61.9 \pm 50.63	48.6 \pm 30.65	30.2 \pm 13.85	F= 4.85	0.001 (S)
Total bilirubin (mg/dL)	0.67 \pm 0.24	1.02 \pm 0.26	3.32 \pm 1.57	4 \pm 2.92	0.91 \pm 0.33	F=19.2 6	<0.00 1 (S)
Serum albumin (gm/dl)	4.03 \pm 0.3	3.77 \pm 0.36	2.86 \pm 0.64	2.51 \pm 0.9 7	4.1 \pm 0.32	F=24.6 6	<0.00 1 (S)
INR	1.27 \pm 0.18	1.24 \pm 0.15	1.67 \pm 0.36	1.77 \pm 0.23	1.16 \pm 0.14	F=21.4 1	<0.00 1 (S)

P<0.05: significant difference**Table (3):-** Serum Golgi protein 73 in all different groups

Variable	Group I (Chronic HCV) (no.=20)	Group II a (Compensated Liver Cirrhosis) (no.=10)	Group II b (Decompensated Liver Cirrhosis) (no.=10)	Group III (HCC) (no.=40)	Group IV (control group) (no.=10)	Test	P
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD		
Serum Golgi protein 73 (ng/ml)	7.16 \pm 2.6	6.84 \pm 2.61	8.16 \pm 3.84	15.55 \pm 14.91	5.3 \pm 2.03	χ^2 = 12.87	0.01 (S)

P<0.05: significant difference**Table (4):-** Correlation between serum Golgi protein 73 and different laboratory parameters among HCC cases

Variable (no.=40)	Serum Golgi protein 73	
	Spearman Correlation coefficient (rho; ρ)	P
HB (gm/dl)	0.04	0.82
WBCs x1000/cmm	0.22	0.17
Platelets x1000/cmm	-0.32	0.04 (S)
AST	0.51	<0.001 (S)
ALT	0.41	0.008 (S)
T. bilirubin	0.45	0.003 (S)
S. albumin	-0.52	<0.001 (S)
INR	0.57	<0.001 (S)
AFP	0.43	0.005 (S)

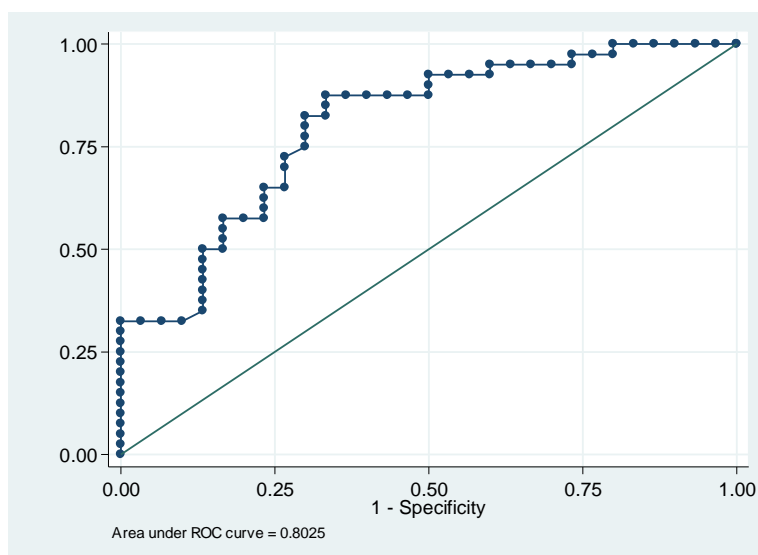
P<0.05: significant difference

Table (5):- Correlation between serum Golgi protein 73 and HCC tumor characteristics

Variable		No. (%)	Serum Golgi protein 73	Test	P
			Mean \pm SD		
Size of focal lesions	≤ 5 cm	29 (72.5%)	13.34 \pm 12.66	Z= 0.76	0.45
	>5cm	11 (27.5%)	21.38 \pm 19.13		
Number of focal lesions	Single	28 (70%)	10.77 \pm 10.56	Z= 2.67	0.008 (S)
	Multiple	12 (30%)	26.71 \pm 17.89		

P<0.05: significant difference**Table (6):-** Correlation between serum Golgi protein 73 and prognostic markers of HCC.

Variable		No. (%)	Serum Golgi protein 73	Test	P
			Mean \pm SD		
Child classification	A	6 (15%)	6.02 \pm 2.94	χ^2 =7.45	0.02 (S)
	B	13 (32%)	9.29 \pm 5.03		
	C	21 (52.5%)	22.15 \pm 17.82		
Okuda staging	I	3 (7.5 %)	7.21 \pm 4.07	χ^2 =10.11	0.006 (S)
	II	22 (55%)	10.29 \pm 11.41		
	III	15(37.5%)	24.93 \pm 16.39		

P<0.05: significant difference**Figure 1:-** serum Golgi protein 73 sensitivity and specificity for diagnosis of HCC.

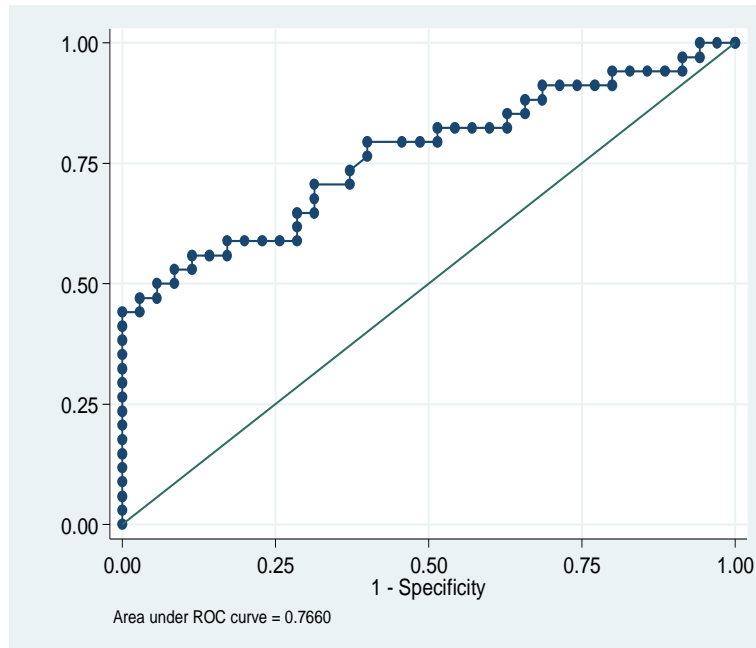


Figure 2:- serum AFP sensitivity and specificity for diagnosis of HCC

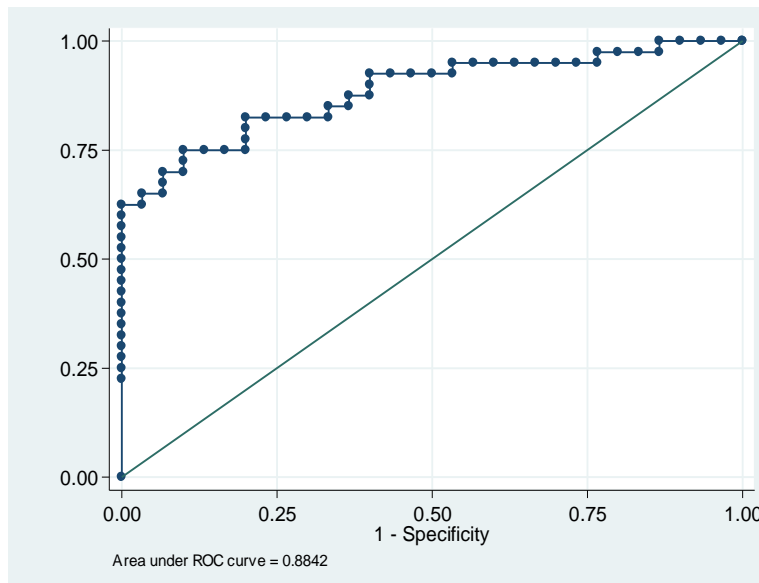


Figure 3:- Combination of serum AFP and serum Golgi protein 73 for diagnosis of HCC.

Discussion:-

The clinical picture of HCC is very variable (Sherlock and Dooley, 2002). Regarding the clinical features: abdominal pain, jaundice, upper GIT bleeding and ascites were the main manifestations in both decompensated liver cirrhosis and HCC groups, That is why screening methods of HCC should be done very thoroughly for its early detection as the clinical features of HCC are nearly similar to patients with cirrhosis, and HCC is not easy to be diagnosed clinically at an early stage (Kew, 2002).

There was a statistical significant difference regarding liver profile tests (PT, INR, total bilirubin, ALT, AST and serum albumin) between both HCC and cirrhotic groups. This agreed with Abdu Allah and his colleagues (2013) who declared that liver function tests distinguished HCC from liver cirrhosis, these results were against to Kew (2002) who documented that liver function tests do not distinguish HCC from cirrhosis.

Panteghini (2006) explained significant marked increase in bilirubin, mild increase in AST and ALT in HCC patients due to the impaired ability of damaged and necrosed hepatocytes to release conjugated bilirubin in addition to the leakage of hepatic enzymes through its inflamed wall. Moreover, serum albumin was significantly lower and PT and INR were significantly higher in HCC patients. This could be attributed to the impaired synthetic ability of the liver for albumin and vitamin K, the co-factor of extrinsic coagulation pathway (**Laffan, 2006 and Johnson, 2006**).

The present results for serum GP73 showed that it revealed higher concentration in HCC patients than those with liver cirrhosis, chronic hepatitis C or healthy controls. This finding was in accordance with that of **Ozkan and his colleagues (2011)** who explained that this increase was due to GP73 over-expression in hepatocytes which appears to be a major contributor to its increased serum.

Additionally, TGF- β is also up-regulated in HCC patient which is an activator of proprotein convertase furin responsible for the cleavage and release of GP73 (**Bachert et al., 2007**). However, this finding was against **Sangiovanni and his colleagues (2007)** and **Gu and his colleagues (2009)** who found non statistical significant difference of serum GP73 between HCC group and liver cirrhosis group. This controversially was explained by **Fimmel and Wright (2009)** who referred this finding to methodological issue as the used antibodies in ELISA technique was not fully humanized which may fail to recognize the serum GP73.

Regarding correlation study using Spearman's rank correlation in HCC patients, serum GP73 showed a significant positive correlation with AST, ALT, total bilirubin and INR, a significant negative correlation with Platelet count and serum Albumin, non significant correlation with age, HB and TLC. This was in agreement with the finding of **Tian and his colleagues (2010)**, who reported that serum GP73 correlated with AST, ALT, albumin. This finding was not in agreement with those of **Riener and his colleagues (2009)** and **Mao and his colleagues (2010)** who demonstrated that serum GP73 was not correlated with serum albumin.

The current study showed that there was a statistical significant positive correlation between serum GP73 with number of hepatic focal lesions, there was non statistical significant correlation between serum Golgi protein 73 with size of focal lesions in liver. These are similar to **Mao and his colleagues (2010)**, **Liu and his colleagues (2017)** who reported that, serum levels of GP73 in patients with HCC were not consistently affected by the tumor sizes and the status of tumor differentiation. **Fimmel and Wright (2009)** showed that increase number of hepatic focal lesions lead to increase cancerous hepatocytes leads to increase of GP73 expression that increased serum GP73.

The current study showed that as regard serum AFP, the diagnostic performance at the cutoff (320ng/mL), showed the diagnostic sensitivity of 79.41%, specificity 60 %, PPV 65.9%, NPV 75 %, AUC 0.766, Parallel to these results of AFP in prediction of HCC, **Hakamada and his colleagues (2008)** reported a sensitivity of 69.3%, specificity 60%. Another two studies by **Trevisani and his colleagues (2001)** and **Gambarin-Gelwan and his colleagues (2000)**, AFP specificity varies from about 76% to 96% and increases with elevated cut off value.

Regarding serum GP73 (ng/mL), a cutoff of 11.1 ng/ mL showed a diagnostic sensitivity of 82.5%, specificity 70%, positive predictive value 78.6%, negative predictive value 75%. The area under the curve (AUC) was 0.8025, these results were similar to those of **Mao and his colleagues (2010)** who found that the diagnostic performance of serum GP73 to discriminate between HCC and liver cirrhosis showed a diagnostic sensitivity 75% and specificity 97% at the cutoff 8.5 relative unite, **Marrero and his colleagues (2005)** reported that sensitivity of 70%, specificity of 86% were found at cut-off value 10ng/ml, **Hu and his colleagues (2010)** reported that the sensitivity 77%, specificity 84% were calculated at cut off value 7.4 ng/ml.

GP73 has excellent diagnostic performance for HCC which agree with **Marrero and his colleagues (2005)**, **Mao and his colleagues (2010)** who recommended the installation of serum GP73 measurement as a standard blood test for HCC and may also used in surveillance of HCC recurrence in post operative management

The current results showed that the combined use of the two markers (AFP, serum GP73) lead to an increase in the sensitivity and specificity to diagnose early HCC to (82.5%, 80%) respectively, result agrees with **Mao and his colleagues (2010)**, **Wang and his colleagues (2009)**.

Conclusion:-

Serum GP73 is a promising sensitive and specific tumor marker that could be added to the current standard tests for diagnosis of HCC in order to detect the disease at an early stage and hence improving the prognosis and overall survival rate of the patient.

Conflict Of Interest:-

The authors declare no conflict of interests.

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