Development of an Immunoradiometric Assay (IRMA) for Binding of an ^{125}I -anti AFP Antibody to Alpha Fetoprotein (AFP) In Gastric Adenocarcinoma And Gastric Lymphoma Tissues

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Abstract

Background: Most of previous studies have used Immunoradiometric assay (IRMA) for quantitative determination of Alpha- fetoprotein (AFP) in the sera of gastric cancer patients, but no works were recorded the analysis of AFP in gastric cancer tissues by IRMA.

The purposes of the present study were to measure AFP concentrations in cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissues by binding to an ^{125}I -anti AFP antibody and to find out the optimum conditions for forming the ^{125}I -anti AFP antibody/AFP complex.

Methods: Tumor tissue samples were taken from 14 patients with gastric adenocarcinoma and 13 patients with gastric lymphoma. Preliminary tests of AFP binding to an ^{125}I -anti AFP antibody were performed. The optimum conditions, including the AFP concentration, ^{125}I -anti AFP antibody concentration, pH, temperature and time of binding the ^{125}I -anti AFP antibody with AFP in cytosolic and nuclear fractions of gastric cancer tissue homogenates were investigated. The stability of the ^{125}I -anti AFP antibody/AFP complex and the AFP recovery were also examined.

Results: Comparison of biochemical studies for AFP in cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates showed that there were differences in AFP concentrations and the optimum conditions of binding with an ^{125}I -anti AFP antibody.

Conclusion: The results revealed that the determination of AFP concentrations in gastric cancer tissues could be carried out by biochemical binding with an ^{125}I -anti AFP antibody. The binding percent values of the ^{125}I -anti AFP antibody/AFP complexes were increased at the optimum conditions.

Keywords: Alpha Fetoprotein (AFP), ^{125}I -anti AFP antibody, Immunoradiometric Assay (IRMA), Gastric Adenocarcinoma and Gastric Lymphoma Tissues.

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Introduction

Alpha Fetoprotein (AFP) was recognized as the first tumour marker in gastrointestinal cancer⁽¹⁾. Several reports have shown that AFP is associated with gastric cancer, with or without metastasis to the liver⁽²⁻⁶⁾.

AFP levels were measured in the sera and tissues of gastric cancer tissues using various techniques⁽⁷⁻¹¹⁾. The majority of prior research applied immunohistochemical (IHC) staining techniques in the detection and classification of AFP location in gastric cancer cells⁽¹²⁻¹⁷⁾. Furthermore, the AFP contents in gastric carcinoma tissues was measured by a radioimmunoassay (RIA) method⁽¹⁸⁾. Immunoradiometric assay (IRMA) was used

for quantitative determination of AFP in the sera of cancer patients⁽¹⁹⁻²¹⁾, but no works were recorded the analysis of AFP in gastric cancer tissues by IRMA.

The objectives of this study were to measure the concentrations of AFP in cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates depending on binding studies with ^{125}I -anti AFP antibody. This study was also aimed to find out the optimum conditions including the AFP concentration, ^{125}I -anti AFP antibody concentration, pH, temperature and time for maximum binding between ^{125}I -anti AFP antibody with AFP in cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates. Finally to evaluate the stability and recovery of the ^{125}I -anti AFP antibody/AFP complex.

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Materials and Methods

Chemicals

All common laboratory chemicals and reagents were analar-grade, and they were obtained from the following companies:-

Immunoradiometric assay kits for AFP were purchased from BYK-Sangtec Diagnostica (Dietzenbach, Germany) and CIS Bio International (Paris, France).

Polyethylene glycol-10000 (PEG-10000), copper sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), bovine serum albumin (BSA), Folin-Ciocalteu reagent, sodium hydroxide (NaOH), diphenylamine, perchloric acid and glacial acetic acid were obtained from BDH (London, UK).

Tris (hydroxymethyl) aminomethane, hydrochloric acid (HCl), sodium carbonate (Na_2CO_3), standard DNA and sodium potassium tartrate (Na K-tartrate) were obtained from Fluka Company (Buchs, Switzerland).

Instruments

The instruments used in this work were as follows: LKB 1270 Rack Gamma II gamma counter (LKB instruments, Turku, Finland), Rotanta cooling centrifuge, 5000 rpm (Hettich, Tuttlingen, Germany), 202MK high-speed centrifuge, 13000 rpm (Sigma, Osterode am Harz, Germany), Thermo Orion pH meter 720 (Thermo Fisher Scientific, Waltham, MA, USA), incubator (Mettmert, Schwabach, Germany), water bath (Mettmert), 210A double beam spectrophotometer (Shimadzu, Kyoto, Japan), Genie 11716 vortex (Bohemia, NY, USA), hot plate magnetic stirrer (Stuart Scientific, Staffordshire, UK), and analytical balance (Sartorius, Goettingen, Germany).

Patients

A total of 27 patients with gastric cancer were included in this study. All patients were admitted for diagnosis and treatment at the following hospitals in Baghdad, Iraq: Baghdad Teaching Hospital (Baghdad Medical City) and Al-Yarmouk Teaching Hospital. Patients with diseases that may interfere with this study were excluded. According to histological studies, patients involved in this study were categorized into the following groups:

Group I: gastric adenocarcinoma, included 14 male patients with age ranging from 50–80 years.

Group II: gastric lymphoma included 13 male patients with age ranging from 20–50 years.

Stomach tumour tissues collection

The tumour tissues were surgically removed from the stomach by gastrectomy. The collected tumour tissues were immediately rinsed with ice-cold saline solution and immersed in the same solution. Then, the samples were collected and stored at -20°C until homogenization.

Preparation of stomach tumour tissue homogenates

The frozen tissues of 14 samples of gastric adenocarcinoma and 13 samples of gastric lymphoma were washed separately with ice-cold normal saline and then weighed. The weight of tumour samples ranged from 2.5–22 g. The tissues samples were minced and pulverized with a scalpel and scissors in a petri-dish placed on ice and then homogenized at 4°C in Tris buffer (0.01 M, pH 7.4) with a ratio of 1:3 (weight: volume) using a manual homogenizer. The crude homogenates were filtered through a nylon mesh sieve to eliminate fibrous connective tissues and then centrifuged at 9000 rpm for 30 min. in a cooling centrifuge at 4°C . The resulting supernatants and pellets were considered cytosolic and nuclear fractions, respectively. Each fraction was used as a source of AFP throughout this study.

Determination of total protein in stomach tumour tissue homogenates

The total protein and DNA content of cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates were determined^{22, 23}.

Preliminary tests of cytosolic and nuclear AFP binding with an ^{125}I -anti AFP antibody in gastric adenocarcinoma and gastric lymphoma tissue homogenates

Four experimental groups were used, including two sets of 14 samples of gastric adenocarcinoma tissues and two sets of 13 samples of gastric lymphoma tissues. One hundred microliters of either cytosolic or nuclear fractions were each incubated with 100 μl of ^{125}I -anti AFP antibody and Tris buffer (0.01 M, pH 7.4) was added to make a total volume of 1 ml. The tubes were incubated at 25°C for 4 hr. After incubation, 500 μl of PEG-10000 solution was added as a precipitating reagent to the tubes and then mixed gently on a vortex mixer at low speed. Following incubation for 1 hr at 25°C , the tubes were centrifuged at 4000 rpm for 1 hr at 4°C . The supernatants were decanted into new tubes, and the amount of bound radioactivity (c.p.m) was counted in a gamma counter for one minute. Two additional tubes containing only 100 μl of ^{125}I -anti AFP antibody (for total radioactivity) were counted.

Calculation

1. The bound fraction (B) represents the radioactivity measured in each tube, i.e., ^{125}I -anti AFP antibody/AFP complex, expressed in c.p.m.
2. Total activity (T) represents the radioactivity measured in the tubes containing ^{125}I -anti AFP antibody only.
3. The (B/T) % ratio for each tube was counted as follows:

$$(B/T) \% = \frac{\text{Sample mean counts (B)}}{\text{Total activity mean count (T)}} \times 100$$

Influence of cytosolic and nuclear AFP concentrations from gastric adenocarcinoma and gastric lymphoma tissue homogenates on ^{125}I -anti AFP antibody binding

Increasing concentrations (18.7, 37.4, 74.8, 149.6, 299.3, and 598.7 μg protein) of cytosolic fractions and the concentrations (7, 15, 30, 60, and 120 μg DNA) of nuclear fractions from 14 samples of gastric adenocarcinoma and 13 samples of gastric lymphoma homogenates at a volume of 100 μl were combined with 100 μl of ^{125}I -anti AFP antibody; each mixture was then adjusted to a final volume of 1 ml by the addition of Tris buffer (0.01 M, pH 7.4). The procedure and calculation were carried out as mentioned in the preliminary tests. The binding percent values (B/T) % were plotted against either increasing concentrations of protein (AFP) or DNA from the cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates.

Effect of ^{125}I -anti AFP antibody concentration on binding with AFP in cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates

One hundred microliter of cytosolic and nuclear fractions (149 μg protein, 15 μg DNA) from 14 samples of gastric adenocarcinoma and 13 samples of gastric lymphoma (291 μg protein, 74 μg DNA) were incubated with different volumes of ^{125}I -anti AFP antibody (10, 30, 50, 70, 90, 100, and 110 μl), and the volumes were then adjusted to 1 ml with Tris buffer (0.01 M, pH 7.4). The procedure and calculation were followed as described in the preliminary tests.

Effect of pH on cytosolic and nuclear AFP binding with an ^{125}I -anti AFP antibody in gastric adenocarcinoma and gastric lymphoma tissue homogenates

The procedure was carried out with Tris buffer (0.01 M) at different pH values (6.8 to 8.2) using the optimum conditions of AFP concentrations and ^{125}I -anti AFP antibody (1.02 mg/ml).

Effects of time and temperature on cytosolic and nuclear AFP binding with an ^{125}I -anti AFP antibody in gastric adenocarcinoma and gastric lymphoma tissue homogenates

This experiment was performed at various temperatures (5, 15, 25, 35, and 45°C) and at different time intervals (60, 120, 180, 240, 300, 360, 420, and 480 min.) on cytosolic and nuclear fractions of the homogenates at the optimum conditions of AFP concentration, ^{125}I -anti AFP antibody and pH values.

Stability of ^{125}I -anti AFP antibody/AFP complex in gastric adenocarcinoma tissue homogenates

This experiment was carried out on cytosolic and nuclear fractions of 14 samples of gastric adenocarcinoma tissues as described in the preliminary tests, except that optimum conditions were used. After evaluating for bound AFP, the ^{125}I -anti AFP antibody/AFP complexes were re-incubated at five different temperatures (5, 15, 25, 35, and 45°C) for a duration between 0 and 6 hr; the bound AFP remaining at each temperature was measured by using a gamma counter. The calculation was conducted as described in the preliminary tests.

Recovery of AFP

This experiment was carried out at optimum conditions for cytosolic and nuclear fractions of AFP from 14 samples of gastric adenocarcinoma and 13 samples of gastric lymphoma tissue homogenates. The experiment was performed in three sets of coated tubes as follows. In the first set, 250 μl of Tris buffer at optimum pH for cytosolic adenocarcinoma and lymphoma tissue homogenates (7.4 or 7.2, respectively) or nuclear adenocarcinoma and lymphoma tissues (6.8 or 7.8, respectively) was dispensed into the coated tubes in addition to 50 μl of each homogenate at optimum protein concentration and 50 μl of unlabelled AFP (900 ng/ml). In the second set, 300 μl of Tris buffer at the respective optimum pH for each tissue homogenate was dispensed into the tubes along with 50 μl of each homogenate. In the third set of tubes, 300 μl of Tris buffer at the respective optimum pH for each tissue homogenate and 50 μl of unlabelled AFP (900 ng/ml) were added to each tube. All tubes from each set were gently mixed on a vortex mixer at low speed. Then, all tubes were incubated at an optimum temperature of 35°C with agitation at 400 rpm; an optimum time of 6 hr was used for cytosolic and nuclear fractions from gastric adenocarcinoma whereas 8 hr and 5 hr incubation durations were used for cytosolic and nuclear fractions from gastric lymphoma, respectively. The contents of the tubes were aspirated as completely as possible and subsequently washed twice with 3 ml of deionized distilled water. To each tube, 300 μl of ^{125}I -anti AFP antibody was added prior to incubation for 30 min. at optimum temperature (35°C) with agitation at 400 rpm. The tubes were washed as described previously. The radioactivity bound to the tube was measured by analysis with a gamma counter.

Calculation

1. The mean c.p.m. was determined for each pair of duplicate tubes from the three experimental sets.
2. The mean c.p.m. from the first experimental set represents the recovered values.
3. The sum of the mean c.p.m. from the second and third experimental sets represent the expected values.
4. The percent recovery can be calculated according to

the following:

$$\text{Recovery \%} = \frac{\text{Recovered values (c.p.m)}}{\text{Expected values (c.p.m)}} \times 100$$

Results

The cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates were used as the sources of AFP in this study. The (B/T) % represents the binding percent of ^{125}I -anti AFP antibody with AFP, and then the concentrations of AFP accordingly.

The preliminary results revealed that the amount of binding percent (B/T) % in nuclear fractions (20.03% and 20.41%) of gastric adenocarcinoma and gastric lymphoma tissue homogenates were higher than those in cytosolic fractions (15.0% and 18.3%) of gastric adenocarcinoma and gastric lymphoma tissues, respectively.

A PEG-10000 solution was used to separate the ^{125}I -anti AFP antibody/AFP complex formed from the unbound particulates.

Figure 1 shows that 149.6 μg and 291.6 μg were the most appropriate concentrations of AFP to generate maximum binding in cytosolic fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates, respectively. While 15 μg and 74 μg were the optimum AFP concentrations in nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates, respectively.

The maximum binding percent was obtained at an ^{125}I -anti AFP antibody concentration of 1.02 mg/ml as revealed in Figure 2. The maximum bindings of AFP with ^{125}I -anti AFP antibody were occurred at a pH of 7.4 and 7.2 in cytosolic fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates, respectively as shown in Figure 3. While, the optimum pH of AFP binding in nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates were 6.8 and 7.8, respectively.

The time course analysis at different temperatures indicated that binding of the ^{125}I -anti AFP antibody to AFP in cytosolic and nuclear fractions is a temperature and time-dependent process. As shown in Figure 4, the maximum binding percent was obtained at 35°C after incubation for 6 hr in cytosolic and nuclear fractions of gastric adenocarcinoma tissue homogenates, whereas incubation for 8 hr and 5 hr was optimal for cytosolic and nuclear fractions of gastric lymphoma tissue homogenates, respectively. The influence of temperature on the stability of the ^{125}I -anti AFP antibody/AFP complex as a function of time was studied. Figure 5 shows the rate of dissociation of the ^{125}I -anti AFP antibody/AFP complexes at

different temperatures. At 5°C and 15°C, approximately 28% and 39% of the complexes were dissociated in cytosolic and nuclear fractions of gastric adenocarcinoma tissue homogenates, respectively. At 35°C, the ^{125}I -anti AFP antibody/AFP complexes in cytosolic and nuclear fractions were more stable than those at other temperatures.

The results summarized in Table 1 indicate that the recovery percentages of cytosolic and nuclear AFP (93.39% and 90.74%) in gastric adenocarcinoma tissue homogenates were greater than the recovery percentages of cytosolic and nuclear AFP (86.41% and 84.60%) in gastric lymphoma tissue homogenates. Thus, the proportional errors of the cytosolic and nuclear AFP in gastric lymphoma tissues were greater than the proportional errors of the cytosolic and nuclear AFP in gastric adenocarcinoma tissues.

Furthermore, the results indicate that AFP concentration can be determined through the binding studies with ^{125}I -anti AFP antibody, while the percent recovery indicates the precision of the method used.

Discussion

According to the previous studies, the presence of AFP in gastric cancer was investigated by immunohistochemical methods that was performed on formalin-fixed paraffin-embedded tissues.

In this study, biochemical studies of AFP binding with ^{125}I -anti AFP antibody were carried out on gastric cancer tissues without embedded in formalin to find out the differences in AFP concentrations and binding properties at cellular and nuclear locations of both gastric adenocarcinoma and gastric lymphoma tissues.

The preliminary results revealed that AFP concentrations in gastric lymphoma tissues were greater than those in gastric adenocarcinoma tissues.

The optimum conditions for binding the ^{125}I -anti AFP antibody with AFP in cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates were investigated. Figure 1 indicated that binding of AFP to ^{125}I -anti AFP antibody increases with increasing amounts of AFP in the homogenates. The decrease in binding percent at high concentrations of cytosolic and nuclear fractions of tissues in the reaction mixtures may be due to conformational changes in AFP and the ^{125}I -anti AFP antibody rather than the formation of reversible, inactive ^{125}I -anti AFP antibody/AFP complex.

In comparison with the preliminary results and using the optimal conditions of AFP concentration, ^{125}I -anti AFP antibody concentration and pH, the binding percent values (B/T) % were increased at optimum temperature and time and reached to the maximum with the increment 24.99%, 15.01%, 24.0% and 17.17% in cytosolic and

Figure 1 Influence of AFP concentrations in cytosolic fractions of gastric adenocarcinoma tissues (●) and gastric lymphoma tissues (■) on binding to ^{125}I -anti AFP antibody.

The figure was plotted at error amount 5% and 1.0 standard deviation.

N= 14 for Gastric adenocarcinoma tissues

N= 13 for gastric lymphoma tissues

The (B/T) % represents the binding percent of ^{125}I -anti AFP antibody with AFP and forming the ^{125}I -anti AFP antibody/AFP complex.

Maximum binding percent of AFP in cytosolic fractions occurred at 149.6 µg and 291.6 µg of gastric adenocarcinoma and gastric lymphoma tissue homogenates, respectively.

Statistical differences between AFP concentrations in gastric adenocarcinoma and gastric lymphoma tissues at the optimum concentrations of AFP were indicated by Standard Error Bars.

Figure 2 Effect of different concentrations of ^{125}I -anti AFP antibody on binding with AFP in cytosolic fractions of gastric adenocarcinoma tissues (●) and gastric lymphoma tissues (■).

The figure was plotted at error amount 5% and 1.0 standard deviation.

N= 14 for Gastric adenocarcinoma tissues

N= 13 for gastric lymphoma tissues

The (B/T) % represents the binding percent of ^{125}I -anti AFP antibody with AFP and forming the ^{125}I -anti AFP antibody/AFP complex.

The maximum binding percent was obtained at an ^{125}I -anti AFP antibody concentration of 1.02 mg/ml.

Standard Error Bars revealed that there was no statistical difference between cytosolic AFP binding in gastric adenocarcinoma and gastric lymphoma at different concentrations of an ^{125}I -anti AFP antibody.

Figure 3 Effect of pH on the binding of ^{125}I -anti AFP antibody with AFP in cytosolic fractions of gastric adenocarcinoma tissues (●) and gastric lymphoma tissues (■).

The figure was plotted at error amount 5% and 1.0 standard deviation.

N= 14 for Gastric adenocarcinoma tissues

N= 13 for gastric lymphoma tissues

The (B/T) % represents the binding percent of ^{125}I -anti AFP antibody with AFP and forming the ^{125}I -anti AFP antibody/AFP complex.

Maximum binding percent occurred at a pH of 7.4 and 7.2 for cytosolic fractions of gastric adenocarcinoma and gastric lymphoma tissues, respectively.

Standard Error Bars indicated that there was statistically difference between AFP binding at a pH 7.2 for cytosolic fractions of gastric adenocarcinoma and gastric lymphoma tissues, respectively.

Figure 4 Effect of time – temperature on the binding of ^{125}I -anti AFP antibody with AFP in cytosolic fractions of gastric adenocarcinoma tissues.

The figure was plotted at error amount 5% and 1.0 standard deviation.

N= 14 for Gastric adenocarcinoma tissues

N= 13 for gastric lymphoma tissues

The (B/T) % represents the binding percent of ^{125}I -anti AFP antibody with AFP and forming the ^{125}I -anti AFP antibody/AFP complex.

The figure indicated that the optimum temperature and time conditions for cytosolic fractions of gastric adenocarcinoma were 35°C and 6 hr, respectively.

The standard error bars of time – temperature curve indicated that there were no significant differences between cytosolic and nuclear AFP in gastric adenocarcinoma and gastric lymphoma tissue homogenates.

Figure 5 Stability of ¹²⁵I-anti AFP antibody/AFP complex at different temperatures in cytosolic fractions of gastric adenocarcinoma tissues.

The figure was plotted at error amount 5% and 1.0 standard deviation.

N= 14 for Gastric adenocarcinoma tissues

The (B/T) % represents the binding percent of ¹²⁵I-anti AFP antibody with AFP and forming the ¹²⁵I-anti AFP antibody/AFP complex.

The figure indicated that the ¹²⁵I-anti AFP antibody/AFP complexes in the cytosolic fractions were more stable at 35°C than those at other temperatures.

The standard error (SE) bars indicated that the stability of the ¹²⁵I-anti AFP antibody/AFP complex was statistically different at 35°C in cytosolic fractions of gastric adenocarcinoma tissue homogenates.

Table 1 Recovery of AFP

Type of AFP	Standard (c.p.m.)	Homogenates (c.p.m.)	Expected* (c.p.m.)	Recovered (c.p.m.)	Recovery %	Proportional error (100-% recovery)
Gastric adenocarcinoma						
Cytosolic AFP	3209	2422	5631	5259	93.39	6.61
Nuclear AFP	3209	3044	6253	5674	90.74	9.26
Gastric lymphoma						
Cytosolic AFP	3209	2076	5285	4567	86.41	13.59
Nuclear AFP	3209	2352	5561	4705	84.60	15.4

* The expected values represent the sum of the mean c.p.m. of homogenates and the standard.

nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates, respectively.

The decrease in binding percent (B/T) % of AFP in cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates with the ^{125}I -anti AFP antibody in time – temperature course might be due to either degradation of AFP or irreversible dissociation of the ^{125}I -anti AFP antibody/AFP complex. At higher temperatures, denaturation and destruction of tertiary structure may occur, leading to loss of activity and conformational changes in the proteins. Heating the solutions to greater than 35°C disrupts the folded structure of the protein by increasing the vibrational and rotational motions of atoms²⁴⁾.

Wu and Knight²⁵⁾ measured the stability of AFP in clinical specimens at different AFP concentrations and temperatures. They concluded that AFP depends on both incubation temperature and AFP concentration. Lantz, et al. reported a significant change in serum AFP concentrations over time²⁶⁾. Uversky, et.al. indicated that AFP denaturation occurs at acidic pH.²⁷⁾ Wang, et.al. also reported that the activities of both antigen and antibody were affected by the acidity of the detection solution²⁸⁾. The tertiary structure of AFP showed that the external hydrophilic surface together with multiple internal hydrophobic molecular clefts enhance ligand binding²⁹⁾.

The validity of the developed method in this study was assessed by calculating the recovery of cytosolic and nuclear AFP that were extracted from gastric adenocarcinoma and gastric lymphoma tissue homogenates.

The recovery of the analyte is a concentration-dependent and the magnitude of the proportional systematic error increases as the analyte concentration of analyte increases^{30, 31)}.

Conclusion

Biochemical studies of ^{125}I -anti AFP antibody binding with AFP in cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates were performed in this study. The preliminary tests for determination of AFP concentrations in cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates gave satisfactory results. The binding percent values of the ^{125}I -anti AFP antibody/AFP complexes were increased at the optimum conditions.

The optimum conditions for the determination of AFP in cytosolic fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates were as follows – AFP concentration: $149.6\text{ }\mu\text{g}$ and $291.6\text{ }\mu\text{g}$ protein, respectively; ^{125}I -anti AFP antibody concentration: 1.02 mg/ml ; pH: 7.4 and 7.2, respectively; temperature: 35°C ; and time: 6 hr and 8 hr, respectively. The optimal conditions for the quantitation of AFP in nuclear fractions of

gastric adenocarcinoma and gastric lymphoma tissue homogenates were as follows: AFP concentration: $15\text{ }\mu\text{g}$ and $74\text{ }\mu\text{g}$ DNA, respectively; ^{125}I -anti AFP antibody concentration: 1.02 mg/ml ; pH: 6.8 and 7.8, respectively; temperature 35°C and time: 6 hr and 5 hr, respectively. The ^{125}I -anti AFP antibody/AFP complex was found to be more stable at 35°C than at other temperatures. The percent recovery indicates the precision of the method used and high concentrations of AFP in these two groups of gastric tumour tissues.

Disclosure Statement:

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References

- 1) Zinser, J. W. (1997). Tumor markers in cancer of the digestive system. *Rev. Gastroenterol. Mex.* 62 (3): 145–8.
- 2) Ishiguro, T., Sugitachi, I., Sakaguchi, H. and Itani, S. (1985). Serum alpha-fetoprotein subfractions in patients with primary hepatoma or hepatic metastasis of gastric cancer. *Cancer* 55 (1): 156–9. DOI: 10.1002/1097-0142(19850101)55:1<156::aid-cncr2820550124>3.0.co;2-e
- 3) Bourreille, J., Metayer, P., Sauger, F., Matray, F. and Fondimare, A. (1970). Existence of alpha feto protein during gastric-origin secondary cancer of the liver. *Presse Med.* 78(28):1277–1278.
- 4) Augustin, G., Jelincic, Z., Tentor, D., Majerovic, M. and Matosevic, P. (2009). Hepatoid adenocarcinoma of the stomach: case report and short notes on immuno histochemical marker. *Acta Gastro-Enterologica Belgica*, Vol. LXXXII.
- 5) Chang, Y. C., Nagasue, N., Abe, S., Kohno, H., Kumar, D. D. and Nakamura, T. (1991). A Fetoprotein producing early gastric cancer with liver metastasis: report of three cases. *Gut* 32(5): 542-545. DOI: 10.1136/gut.32.5.542
- 6) Chang, Y. C., Nagasue, N., Abe, S., Taniura, H., Kumar, D. D. and Nakamura, T. (1992). Comparison between the clinicopathologic features of AFP-positive and AFP-negative gastric cancers. *Am. J. Gastroenterol.* 87(3): 321–325.
- 7) McIntire, K. R., Waldmann, T. A., Moertel, C. G. and Go, V. L. (1975). Serum alpha-fetoprotein in patients with neoplasms of the gastrointestinal tract. *Cancer Res.* 35(4): 991–996.
- 8) Mehlman, D. J., Bulkley, B. H. and Wiernik, P. H. (1971). Serum alpha-1-fetoglobulin with gastric and prostatic carcinomas. *N. Engl. J. Med.* 285(19): 1060–1. DOI: 10.1056/NEJM197111042851907
- 9) Sajid, K.M. (1999). Correlation of tumor makers (tpsa, fpsa, afp, and beta-hcg) irma with chemiluminescence assay results. *Nucleus (Islamabad)*, 36(1–2), 99–102.
- 10) Chun, H. and Kwon, S. J. (2011). Clinicopathological characteristics

- of alpha-fetoprotein-producing gastric cancer. *Journal of gastric cancer*, 11(1), 23–30. <https://doi.org/10.5230/jgc.2011.11.1.23>
- 11) Er-Bao Chen, Yi-Chou Wei, Hai-Ning Liu, Cheng Tang, Meng-Ling Liu, Ke Peng, and Tianshu Liu. (2019). Hepatoid Adenocarcinoma of Stomach: Emphasis on the Clinical Relationship with Alpha-Fetoprotein-Positive Gastric Cancer. *BioMed Research International*. Volume 2019, Article ID 6710428, 9 pages. <https://doi.org/10.1155/2019/6710428>
 - 12) Fan, Z., Van de Rijn, M., Montgomery, K. and Rouse, R.V. (2003). Hep Par 1 Antibody Stain for the Differential Diagnosis of Hepatocellular Carcinoma: 676 Tumors Tested Using Tissue Microarrays and Conventional Tissue Sections. *Mod Pathol*. 16(2): 137–144. <https://doi.org/10.1097/01.MP.0000052103.13730.20>
 - 13) Maritra, A., Murakata, L. A. and Albores-Saavedra, J. (2001). Immunoreactivity for hepatocyte paraffin 1 antibody in hepatoid adenocarcinomas of the gastrointestinal tract. *Am J Clin Pathol*. 115(5): 689–694.
 - 14) Tsung, S. H. (2017). Literature Review of Gastric Cancer Producing Alpha-fetoprotein. *Gastrointest Cancer Res Ther*. 2(2): 1019.
 - 15) Koyama, S., Ebihara, T. and Osuga, T. (1987). Histologic and immunohistochemical studies of alpha-fetoprotein (AFP)-producing gastric carcinoma. *Gastroenterologia Japonica*. 22 (4): 419–427.
 - 16) Kato, T., Takahashi, H., Ida, Y., Watanabe, Y., Sato, Y. and Takeda B. (1993). Immunocytochemical studies of AFP producing gastric cancer-cytomorphology and characteristics of AFP positive cells. *Rinsho Byori (Jpn. J. Clin. Pathol.)* 41(9): 1024–1030.
 - 17) Kodama, T., Kameya, T., Hirota, T., Shimosato, Y., Ohkura, H., Mukojima, T. and Kitaoka, H. (1981). Production of alpha-fetoprotein, normal serum proteins, and human chorionic gonadotropin in stomach cancer: histologic and immunohistochemical analyses of 35 cases. *Cancer* 48 (7): 1647–55. DOI: 10.1002/1097-0142(19811001)48:7<1647::aid-cncr2820480729>3.0.co;2-v
 - 18) Kitaoka, H., Hattori, N., Mukojima, T., Ohkura, H., Nakayama, N. and Okada, H. (1973). Alpha-fetoprotein content in tissues from patients with gastric cancer. *Tumor Res*. 8: 171–177.
 - 19) Maussier, M. L., Valenza, V., Schinco, G. and Gallv, G. (1990). AFP, CEA, CA 19-9 and TPA in Hepatocellular Carcinoma. *The International Journal of Biological Markers* 5(3): 121–126. <https://doi.org/10.1177/172460089000500304>
 - 20) Andreev, A. V., Grigor'eva Elu and Gusev, A. I. (1993). Analytical characteristics of commercial IRMA-AFP kit for determination of human alpha-fetoprotein. *Klin Lab Diagn*. 3: 54–8. <https://www.ncbi.nlm.nih.gov/pubmed/8963541>
 - 21) Kuusela, P., Haglund, C., Roberts, P. J. and Jalanko, H. (1987). Comparison of CA-50, a new tumour marker, with carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) in patients with gastrointestinal diseases. *British Journal of Cancer* 55(6): 673–676. <https://doi.org/10.1038/bjc.1987.137>
 - 22) Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. 193(1): 265–75.
 - 23) Burton, K. (1956). A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J*. 62 (2): 315–323. DOI: 10.1042/bj0620315
 - 24) Price, N. C. and Stevens, L. (1989). *Fundamentals of Enzymology*; 2nd ed., Oxford University Press, New York, pp. 125.
 - 25) Wu, J. T. and Knight, J. A. (1985). In-vitro stability of human alpha-fetoprotein. *Clin Chem*. 31(10):1692-1697. <https://doi.org/10.1093/clinchem/31.10.1692>
 - 26) Lantz, M. E., O'Brien, W. F. and Williams, M. C. (1995). The effect of sample preparation and storage on maternal triple-marker screening. *Obstetrics and Gynecology* 85(6): 919–23. DOI: 10.1016/0029-7844(95)00015-j
 - 27) Uversky, V. N., Kirkitaдзе, M. D., Narizhneva, N. V., Potekhin, S. A., Tomashevski, A. Yu. (1995). Structural properties of alpha-fetoprotein from human cord serum: the protein molecule at low pH possesses all the properties of the molten globule. *FEBS Lett*. 364(2): 165–167.
 - 28) Wang, K., Wei, T., Tu, W., Han, M. J. and Dai, Z. (2013). Gold-antibody nanocomposite thin film fabricated by a liquid-liquid interface technique and its application for the sensitive immunoassay of alpha-fetoprotein. *Anal. Methods* 5(8): 1909–1914. <https://doi.org/10.1039/C3AY26541B>
 - 29) Mizejewski, G. J. (2019). Protein Binding and Interactions with AlphaFetoprotein (AFP): A Review of Multiple AFP Cell Surface Receptors, Intracytoplasmic Binding, and InterMolecular Complexing Proteins. *J Mol Cell Biol Forecast*. 2019; 2(1): 1016.
 - 30) Thompson, M., Ellison, S., Fajgelj, A., Willetts, P. and Wood, R. (1999). Harmonised Guidelines for the Use of Recovery Information in Analytical Measurement. *Pure & Appl. Chem*. 71(2): 337–348.
 - 31) Westgard, J. O. and Quam, E. F. (2019). Interference and Recovery Experiments. Retrieved from <https://www.westgard.com/lesson27.htm#6> (Accessed on April 21, 2019).