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Development of a Radioreceptor Assay for Determination of Alpha-fetoprotein (AFP) Receptors in Gastric Adenocarcinoma and Gastric Lymphoma Tissues

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Abstract

Previous studies have utilized different techniques to examine the presence of alpha-fetoprotein (AFP) receptors in tumour tissues. However, none of these studies has investigated the cytosolic and nuclear AFP receptors in gastric adenocarcinoma and gastric lymphoma tissues using radioreceptor assay studies. To address this gap, a radioreceptor assay was developed to determine the AFP receptor concentrations in homogenates of these tissues and optimize the conditions of labelled AFP binding with its cytosolic and nuclear receptors, which include pH, temperature, time of binding, and ^{125}I-AFP concentration. The study revealed the presence of AFP receptors in gastric adenocarcinoma more than those in gastric lymphoma tissues and indicated that the binding efficiency of AFP receptors with the ^{125}I-AFP in nuclear fractions was higher than those in cytosolic fractions for both tissue types. The developed radioreceptor assay represents a novel approach for the detection and quantification of cytosolic and nuclear AFP receptors in these types of tissues and could be recommended for assessing AFP receptor concentrations in gastric cancer tissues as a method for early cancer detection. This technique might aid the design of a novel cancer detection and treatment approach using AFP receptor-based nanoparticles and reveal novel mechanisms of AFP-receptor antitumour immune responses in the future.

Keywords: Radioreceptor assay; Alpha-fetoprotein (AFP); AFP receptors; ^{125}I-AFP; Gastric adenocarcinoma; Gastric lymphoma.

1. Introduction

Alpha-fetoprotein (AFP) is a glycoprotein tumour marker with ontogenic and oncogenic growth that is found during normal foetal development or in cases of cancerous tumours [1,2]. The AFP gene is located on chromosome 4q25 [3]. Tumour-based AFP is an unmutated form of foetal AFP; there is a slight difference in the structures of the two forms due to glycosylation [4,5]. AFP is composed of three domains and contains subdomains that may function as dimerization interfaces with nuclear and cytosolic receptors, as well as cofactors and inhibitors [6,7].

Several studies have identified and described different proteins that bind to AFP in various parts of cells. AFP can be taken up by cells through receptor-mediated endocytosis and transmembrane passage via specific cell surface receptors [7-10]. Mizejewski [11] emphasized the importance of intracytoplasmic AFP binding proteins, including factors related to nuclear receptors, DNA repair, cell cycle checkpoints, apoptosis, and caspases.

The AFP receptors are particularly noteworthy and represent a promising target for cancer treatment. These receptors are oncofoetal antigens that are found on the surface of embryonic, foetal, and tumour cells, but are not present on normal mammalian cells [12]. While AFP receptor expression in non-proliferating cells is generally low, tumour cells from various origins such as mouse mammary carcinoma, human breast cancer, neuroblastoma, lymphoma, hepatoma, and adenocarcinoma, can display anywhere from several hundred to one million AFP receptors per cell [13-16].

Various techniques have been employed to study AFP receptors, including cytochemical and immunological methods along with in situ hybridization [17],

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light and electron microscopy, laser scanning confocal microscopy in the reflection mode [18], microscopic autoradiography and sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) autoradiography [19], fluorescence-activated cell sorting [13], nitrocellulose blotting assay [15], immunohistochemical staining [20], competitive radioimmunoassay [21], and flow cytometry using an anti-AFP antibody and magnetic resonance molecular probes [22].

In this study, we developed a radioreceptor assay to detect AFP receptors in homogenized gastric cancer tissues, which has not been done in previous research. The aims were to identify cytosolic and nuclear AFP receptors in gastric adenocarcinoma and gastric lymphoma tissue homogenates, and optimize the conditions—including AFP receptor concentration, ^{125}I-AFP concentration, pH, temperature, and time—to maximize binding between ^{125}I-AFP and AFP receptors.

2. Materials and Methods

The study was approved by the Department of Chemistry at the College of Science at Mustansiriyah University in collaboration with the Department of Chemistry at the College of Science at the University of Baghdad. This research was performed at the research laboratory of Prof. Dr. Sami Al-Mudhaffar at the Department of Chemistry in the College of Science, University of Baghdad and at the research laboratory of the Chemistry Department at the College of Science in Mustansiriyah University in Baghdad, Iraq in compliance with the laboratory health and safety protocols.

2.1 Materials

The laboratory chemicals and reagents used in this study were purchased from various companies: AFP radioimmunoassay kit (Incstar Corporation, Stillwater, MN, USA), hydrochloric acid (HCl) (Fluka Company, Buchs, Switzerland), tris (hydroxymethyl) aminomethane (Fluka Company, Buchs, Switzerland), sodium chloride (NaCl) (Fluka Company, Buchs, Switzerland), sodium carbonate (Na_2CO_3) (Fluka Company, Buchs, Switzerland), sodium potassium tartrate (Fluka Company, Buchs, Switzerland), and standard DNA (Fluka Company, Buchs, Switzerland). Bovine serum albumin (BSA) (BDH Company, London, UK), copper sulfate pentahydrate ($CuSO_4.5H_2O$) (BDH Company, London, UK), glacial acetic acid (BDH Company, London, UK), sodium hydroxide (NaOH) (BDH Company, London, UK), Folin-Ciocalteu (BDH Company, London, UK), diphenylamine (BDH Company, London, UK), perchloric acid (BDH Company, London, UK), and dithioerythritol (BDH Company, London, UK).

2.2 Instruments

The following instruments were utilized in this research: LKB 1270 Rack Gamma II gamma counter, 202MK high-speed centrifuge (Sigma Company, Germany), Rotanta cooling centrifuge (Hettich Company, Germany), Memmert incubator and water bath, Thermo Orion pH meter 720 (Thermo Fisher Scientific Company, USA), Genie vortex11716 (Bohemia, USA), 210A double beam spectrophotometer (Shimadzu Company, Japan), analytical balance (Sartorius Company, Germany) and scientific hot plate magnetic stirrer (Stuart Company, Staffordshire, UK).

2.3 Patients

This study recruited a total of twelve individuals with gastric cancer who sought medical care and treatment at two hospitals in Baghdad, Iraq: Al-Yarmouk Teaching Hospital and Baghdad Teaching Hospital (Baghdad Medical City). Patients with comorbidities were excluded from the study. Based on histopathological analysis, the tumours were divided into two groups:

- **Group I**: Seven male patients with gastric adenocarcinoma, with ages ranging from 50 to 80 years.
- **Group II**: Five male patients with gastric lymphoma, with ages ranging from 20 to 50 years.

2.4. Methods

2.4.1. Stomach tumour tissue collection:

The gastrectomy procedure was used to surgically extract tumour tissues from the stomach. After removal, the malignant tissues were washed with a cold saline solution and then stored in the same solution at a temperature of -20^{o}C until they were homogenized [23].

2.4.2 Preparation of stomach tumour tissue homogenates:

The cytosolic and nuclear fractions of homogenized gastric adenocarcinoma and gastric lymphoma tissues were prepared as previously described [23] and utilized as a source of AFP receptors for this investigation. Briefly, the tumor samples were homogenized on ice in Tris buffer (0.01M, pH 7.4) with a ratio (1 tissue weight :3 buffer volume) using manual homogenizer. Then the crude homogenates were filtered and centrifuged at 9000 rpm for 30 min. The supernatant contained the cytosolic components, while the pellet contained the nuclear fractions.

2.4.3 Determination of total protein and DNA contents in stomach tumour tissue homogenates:

The measurement of the total protein and DNA levels in the cytosolic and nuclear fractions of gastric

adenocarcinoma and gastric lymphoma tissue homogenates was conducted according to the methods of Lowry et al. [24] and Burton [25].

2.4.4 Preliminary tests of ^{125}I-AFP binding with its cytosolic and nuclear receptors in gastric adenocarcinoma and gastric lymphoma tissue homogenates:

The detection of cytosolic and nuclear AFP receptors in gastric adenocarcinoma and gastric lymphoma tissue homogenates was achieved by assessing total binding and non-specific binding using the conditions described in section 2.4.5. All experiments were performed in duplicate tubes.

2.4.5 Detection of cytosolic and nuclear AFP receptors of gastric cancer tissue homogenates:

Four experimental groups were utilized, consisting of two groups of gastric adenocarcinoma tissue homogenates and two groups of gastric lymphoma tissue homogenates. For each group, 100µl of either cytosolic or nuclear fractions was added and then mixed with 100µl of ^{125}I-AFP. Tris buffer (0.01M, pH 7.4) was added to reach a total volume of 1ml. The mixture was incubated for 4 hours at 25°C. To measure non-specific binding, the same incubation mixture was prepared, but a 9-fold excess of unlabelled AFP was added as a competitor. Following incubation, 500 µl of AFP precipitating reagent was added, and the tubes were mixed on a vortex mixer at low speed. The tubes were incubated for 1 hour at 25°C, then centrifuged at 4000 rpm for 1 hour at 4°C. The supernatants were transferred to new tubes, and the levels of bound and free radioactivity were evaluated using a gamma counter. Two additional tubes containing only 100 µl of ^{125}I-AFP were counted to determine total radioactivity. All experiments were conducted in duplicate, and the calculations were performed as follows:

1. Total binding (TB) represents the amount of radioactivity bound to the particulate fraction (expressed in c.p.m.) in the absence of unlabelled AFP.
2. The radioactivity (expressed in c.p.m.) measured in the tubes containing labelled AFP and an excess of unlabelled AFP represented the non-specific binding (NSB).
3. Specific binding (SB) (c.p.m.) was calculated by subtracting the radioactivity (c.p.m.) obtained in the presence of unlabelled AFP from the radioactivity produced when no unlabelled AFP was present.

SB (c.p.m.) = TB (c.p.m.) – NSB (c.p.m.)

4. The percentage of specific binding (SB%) is calculated using the following equation:

$$\mathbf{SB\%} = \frac{\mathbf{SB}}{\mathbf{T}} \mathbf{x\,100}$$

Where: -

SB: Specific binding (c.p.m.).

T: Total radioactivity of ^{125}I-AFP (c.p.m.).

2.4.6 Effect of cytosolic and nuclear AFP receptor concentrations in gastric adenocarcinoma and gastric lymphoma tissue homogenates on the binding with ^{125}I-AFP

One hundred microlitres of cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates containing various quantities (10.1, 20.3, 40.6, 81.2, 162.5, 325, and 650µg for cytosolic fractions, and 12.5, 25, 50, 100, and 200µg for nuclear fractions) were mixed with 100µl of ^{125}I-AFP. The mixtures were then brought to a final volume of 1 ml with Tris buffer (0.01M, pH 7.4). The specific binding percentage (SB%) was calculated as described in the preliminary test experiment and plotted against the increasing concentrations of AFP receptors in the cytosolic or nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissues homogenates.

2.4.7 Influence of ^{125}I-AFP concentrations on binding with cytosolic and nuclear AFP receptors in gastric adenocarcinoma and gastric lymphoma tissue homogenates

In this experiment, various volumes (10, 30, 50, 70, 90, 100, and 110µl) of ^{125}I-AFP were mixed with 100 µl of cytosolic fractions of gastric adenocarcinoma (40.6 µg/ml) or gastric lymphoma (18.7 µg/ml) and made up to a final volume of 1 ml with Tris buffer (0.01M, pH 7.4). Similarly, different volumes of ^{125}I-AFP were mixed with 100 µl of nuclear fractions of gastric adenocarcinoma (25µg/ml) or gastric lymphoma (23.7µg/ml), and the volume was adjusted to 1 ml with Tris buffer (0.01M, pH 7.4). The calculation procedure from the preliminary test experiment was repeated, and the resulting SB% values were plotted against the concentrations of ^{125}I-AFP.

2.4.8 Influence of pH on ^{125}I-AFP binding with cytosolic and nuclear AFP receptors in gastric adenocarcinoma and gastric lymphoma tissue homogenates

One hundred microlitres of cytosolic fractions from gastric adenocarcinoma (40.6µg/ml) and gastric lymphoma (18.7µg/ml) were mixed with 100µl of ^{125}I-AFP at a concentration of 14.28×10^{-10} M. In addition, 100µl of nuclear fractions from gastric adenocarcinoma (25 µg/ml) or gastric lymphoma (23.7µg/ml) were mixed with 100µl of ^{125}I-AFP at the same concentration. The final volume of each mixture was adjusted to 1ml with Tris buffer (0.01M) with various pH values ranging from 6.8 to 8.2. The procedure and calculations were repeated according to the preliminary test experiment. The values of SB% were plotted against the different pH values.

2.4.9 Time – temperature course of ^{125}I-AFP binding with its cytosolic and nuclear AFP receptors in gastric adenocarcinoma and gastric lymphoma tissue homogenates:

A total of four mixtures, consisting of cytosolic and nuclear fractions from gastric adenocarcinoma (40.6µg/ml and 25µg/ml, respectively) or gastric lymphoma (18.7µg/ml and 23.75µg/ml, respectively), were incubated with 100µl of ^{125}I-AFP (14.28 x10^{-10} M), with or without a 9-fold excess of unlabelled AFP. The volumes of the mixtures were made up to 1 ml with Tris buffer (0.01M) at their respective optimum pH values. For cytosolic and nuclear fractions of gastric adenocarcinoma, the optimum pH values were 8 and 7.6, respectively. While for cytosolic and nuclear fractions of gastric lymphoma, the optimum pH values were 7.8 and 7.4, respectively. The tubes were then incubated at different temperatures (5, 15, 25, 35, and 45°C) and time intervals (60, 120, 180, 240, 300, 360, 420, and 480 min) with the same protocol and calculation as described in the preliminary test experiment.

3. Results and Discussion

In our previous study, the binding of ^{125}I-anti AFP antibody with AFP in the cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates was investigated through biochemical studies. The initial tests conducted to determine AFP concentrations in these fractions yielded satisfactory results.

The study found that the binding percentage values of the ^{125}I-anti AFP antibody/AFP complexes increased under optimal conditions [23]. The optimal conditions for determining AFP concentrations in the cytosolic fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates were determined to be: AFP concentrations of 149.6µg/ml and 291.6µg/ml protein, respectively; ^{125}I-anti AFP antibody concentration of 1.02 mg/ml; pH of 7.4 and 7.2, respectively; a temperature of 35°C; and incubation time of 6 hr and 8 hr, respectively. Meanwhile, the optimal conditions for quantifying AFP in the nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates were found to be: AFP concentration of 15µg/ml and 74µg/ml DNA, respectively; ^{125}I-anti AFP antibody concentration of 1.02 mg/ml; pH of 6.8 and 7.8, respectively; a temperature of 35°C; and time of 6 h and 5 h, respectively [23].

The study also revealed that the ^{125}I-anti AFP antibody/AFP complex was more stable at 35°C compared to other temperatures. The precision of the method used was indicated by the percentage recovery, which was high in these two groups of gastric tumour tissues that contain high concentrations of AFP [23].

No prior study in the literature has investigated the cytosolic and nuclear AFP receptors in gastric adenocarcinoma and gastric lymphoma tissues by radioreceptor assay studies. Therefore, a radioreceptor assay technique was developed in this study to quantify cytosolic and nuclear AFP receptors in gastric adenocarcinoma and gastric lymphoma tissue homogenates and optimize binding of the labelled AFP with their cytosolic and nuclear receptors.

Thus, AFP receptors were extracted from cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates. The SB% indicated the specific binding percentage of ^{125}I-AFP with AFP receptors obtained from these tissues, which reflects the concentrations of AFP receptors present. As per the preliminary findings presented in Table 1, the concentrations of AFP receptors were higher in cytosolic and nuclear fractions of gastric lymphoma tissues compared to those in gastric adenocarcinoma tissues. Furthermore, the concentration of nuclear AFP receptors was higher than that of cytosolic AFP receptors in both gastric adenocarcinoma and gastric lymphoma tissues.

In order to determine the optimal concentrations of AFP receptors for binding to 125I-AFP in the homogenates, different concentrations of cytosolic and nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissues homogenates were incubated with 100µl of 125I-AFP.

As indicated in Figure 1, the concentrations of AFP receptors that resulted in the highest binding efficiency in cytosolic fractions of gastric adenocarcinoma and gastric lymphoma tissues homogenates were determined to be 40.6 µg/ml and 18.7 µg/ml, respectively.

Table 1. Preliminary testing of ^{125}I-AFP specific binding to its receptors in gastric adenocarcinoma and gastric lymphoma tissues.

Group	No. of cases	SB%
Gastric adenocarcinoma	7(58%)*	
Cytosolic receptor		3.0
Nuclear receptor		4.1
Gastric lymphoma	5(42%)*	
Cytosolic receptor		4.5
Nuclear receptor		5.2

*****The numbers in parentheses represent the incidence of AFP receptors in gastric adenocarcinoma and gastric lymphoma tissues.

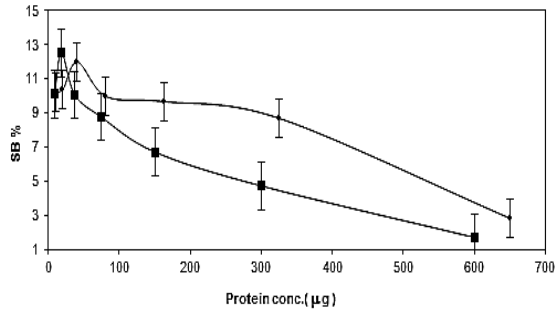

Figure 1. Influence of cytosolic AFP receptor concentrations on the binding with ^{125}I-AFP in: gastric adenocarcinoma tissues (●); gastric lymphoma tissues (■). The graph was generated with an error margin of 5% and a standard deviation of 1.0. The sample sizes for gastric adenocarcinoma and gastric lymphoma tissues were 7 and 5, respectively. The SB% measured the percentage of specific binding of ^{125}I-AFP with its cytosolic receptor. The highest percentage of specific binding of AFP receptors in cytosolic fractions was observed at 40.6 µg/ml and 18.75 µg/ml of homogenates from gastric adenocarcinoma and gastric lymphoma tissues, respectively. Standard Error Bars revealed that there was no statistical difference between AFP receptor binding in cytosolic fractions of gastric adenocarcinoma and gastric lymphoma tissues at the optimum concentrations of cytosolic AFP receptors.

As shown in Figure 2, the optimal concentrations of AFP receptors for AFP receptor binding in nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissue homogenates were 25µg/ml and 23.75µg/ml, respectively

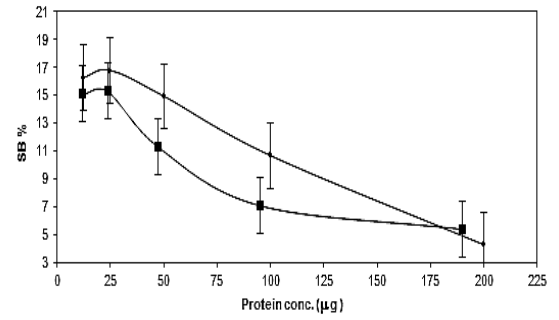

Figure 2. Influence of nuclear AFP receptor concentrations on the binding with ^{125}I-AFP in: gastric adenocarcinoma tissues (●); gastric lymphoma tissues (■). The graph was generated with a 5% error margin and a standard deviation of 1.0. The sample size was N=7 for gastric adenocarcinoma tissues and N=5 for gastric lymphoma tissues. The SB % indicated the percentage of specific binding of ^{125}I-AFP with its nuclear receptor. The highest level of specific binding of AFP receptors in nuclear fractions was observed at 25µg/ml and 23.75µg/ml for gastric adenocarcinoma and gastric lymphoma tissues, respectively. The Standard Error Bars indicated that there was no significant difference in the binding of AFP receptors in the nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissues at the optimal AFP receptor concentrations.

The specific binding percentage of cytosolic and nuclear fractions of tumour tissues tends to decrease at high concentrations, possibly because AFP molecules can bind in an improper orientation to the binding sites on the AFP receptors and thereby reduce the specific binding percentage. Alternatively, this trend may be due to changes in the conformation of AFP receptors at high concentrations causing a decrease in the specific binding percentage with^{125}I -AFP.

According to the literature, a soluble form of the AFP receptor (RECAF) was detected in cytosol fractions of the MCF-7 human breast cancer cells using a nitrocellulose blotting assay and was shown to rapidly bind endogenous AFP [15]. Tcherkassova et al. (2017) measured the concentration of AFP receptor in homogenates of the estrogen-dependent breast carcinoma T47D and colorectal adenocarcinoma (SW620), and in hepatocellular carcinoma (HepG2) cell extracts using a solid-phase chemiluminescent assay. They found that the concentration of AFP receptors in T47D (9152 Units/mg) was greater than in HepG2 (4865 Units/mg), while the concentration of AFP receptor in SW620 was the lowest (2839 Units/mg) compared to the other cell lines [26].

Figures 3 and 4 illustrate how ^{125}I-AFP binding to AFP receptors in homogenized gastric adenocarcinoma and gastric lymphoma tissues varied with different concentrations of ^{125}I-AFP. The highest specific binding percentage occurred at a ^{125}I-AFP concentration of 14.28 x 10^{-10} M.

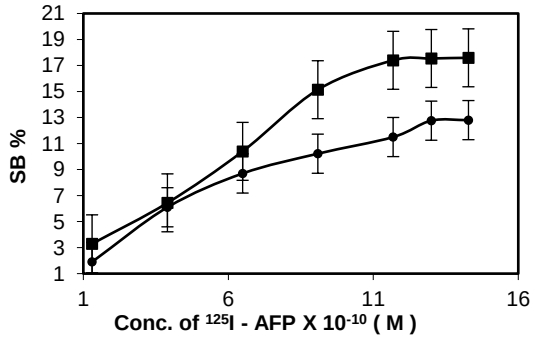

Figure 3. Effect of different concentrations of ^{125}I-AFP on binding with its cytosolic receptors in: gastric adenocarcinoma tissues (●); gastric lymphoma tissues (■). The graph was generated using a 5% error margin and a standard deviation of 1.0. The data points for gastric adenocarcinoma tissues were based on 7 observations, while those for gastric lymphoma tissues

were based on 5 observations. The y-axis values represented the specific binding percentage of ^{125}I-AFP with its cytosolic receptors. For both gastric adenocarcinoma and gastric lymphoma tissues, the maximum specific binding percentage was observed at a ^{125}I-AFP concentration of 14.28 x 10^{-10} M. The statistical analysis showed significant differences between the concentrations of AFP receptors in the two types of tissues at this optimal concentration of ^{125}I-AFP.

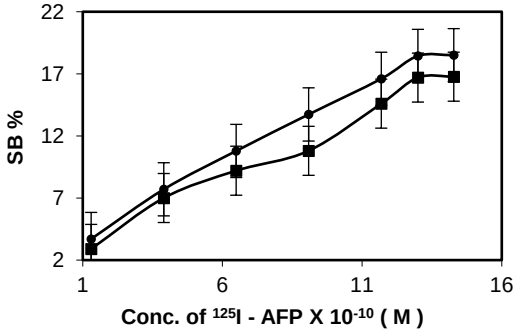

Figure 4. Effect of different concentrations of ^{125}I-AFP on binding with its nuclear receptors in: gastric adenocarcinoma tissues (●); gastric lymphoma tissues (■). The graph displayed data with a 5% error margin and a standard deviation of 1.0. The number of observations for gastric adenocarcinoma tissues was 7, while for gastric lymphoma tissues it was 5. The y-axis values represented the specific binding percentage of ^{125}I-AFP with its nuclear receptors. For both gastric adenocarcinoma and gastric lymphoma tissues, the maximum specific binding percentage was observed at a ^{125}I-AFP concentration of 14.28 x 10^{-10} M. Standard Error Bars showed that there was no statistically significant difference in the binding of nuclear AFP receptors between gastric adenocarcinoma and gastric lymphoma tissues at different concentrations of ^{125}I-AFP.

It was noticed that the specific binding (SB%) increased when ^{125}I-AFP increased, forming the maximum percentages of ^{125}I-AFP/AFP receptor complexes. The specific binding percentage then decreased with further increases in ^{125}I–AFP concentration as the binding sites on the AFP receptors reached saturation with ^{125}I–AFP and no more ^{125}I-AFP/AFP receptor complexes were formed. The results showed that the specific binding between ^{125}I-AFP and its receptor is mainly dependent on the concentrations of the AFP receptors and the labelled AFP in the reaction mixture.

Our study investigated how pH affects the binding of ^{125}I-AFP with its receptors in the cytosolic and nuclear fractions of homogenized gastric adenocarcinoma and gastric lymphoma tissues. Tris buffer solutions with pH values ranging from 6.6 to 8.2 were used. According to Figure 5, the highest binding occurred at a pH of 8.0 and 7.8 for cytosolic fractions of gastric adenocarcinoma and gastric lymphoma tissues homogenates, respectively. Figure 6 shows that the optimal pH values for AFP receptor binding with ^{125}I-AFP in nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissues homogenates were 7.6 and 7.4, respectively.

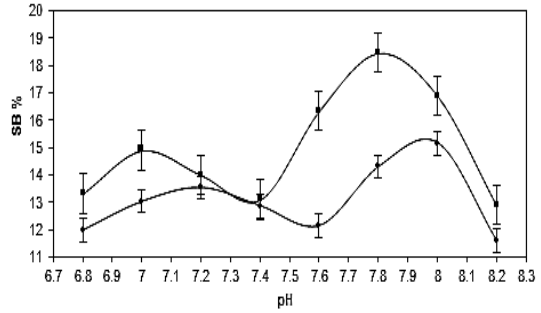

Figure 5. Effect of different pH on the binding of ^{125}I -AFP with its cytosolic receptors in: gastric adenocarcinoma tissues (●); gastric lymphoma tissues (■). The specific binding percentage (SB%) of ^{125}I-AFP with its cytosolic receptors was represented in a figure with error bars showing 5% and 1.0 standard deviation. The study used 7 gastric adenocarcinoma tissues and 5 gastric lymphoma tissues. The maximum SB% for cytosolic AFP receptors occurred at pH 8.0 and 7.8 for gastric adenocarcinoma and gastric lymphoma tissues homogenates, respectively. The Standard Error Bars indicated that there was a statistically significant difference between AFP receptor binding at the optimal pH for cytosolic fractions of gastric adenocarcinoma and gastric lymphoma tissues.

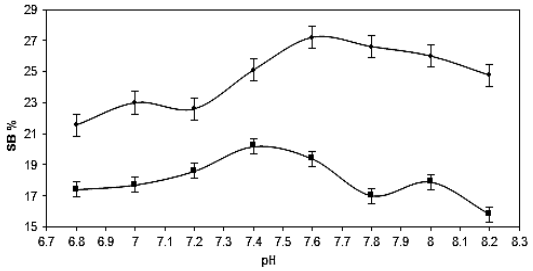

Figure 6. Effect of different pH on the binding of ^{125}I –AFP with its nuclear receptors in: gastric adenocarcinoma tissues (●); gastric lymphoma tissues (■). The chart was generated with an error margin of 5% and a standard deviation of 1.0. The sample sizes were N=7 for gastric adenocarcinoma tissues and N=5 for gastric lymphoma tissues. The SB% is the percentage of specific binding of ^{125}I-AFP with nuclear receptors. The maximum percentage of specific binding of nuclear AFP receptors occurred at pH 7.6 and 7.4 for gastric adenocarcinoma and gastric lymphoma tissues homogenates, respectively. The Standard Error Bars suggested that there was a statistically significant difference in AFP receptor binding at the optimal pH for nuclear fractions of gastric adenocarcinoma and gastric lymphoma tissues.

Mizejewski (2015) described a consecutive series of leucine zipper (heptad) repeats in AFP molecules which have potential heterodimerization activity for the formation of protein-to-protein interactions [5].

According to the literature, AFP adopts a variety of conformational forms and has antigenic determinant sites on its polypeptide chain [27]. It has three peptide domains (I, II, and III) in a secondary structure form that results from several intermolecular loops mediated by 16 disulfide bonds. The disulfide bridges produce a helical tertiary molecular structure that forms a V-shape, as determined using X-ray crystallography. The tertiary structure of the AFP molecule revealed an external hydrophilic surface and several hydrophobic pockets which are located between domain I and III. These hydrophobic pockets can bind covalently to hydrophobic ligands such as drugs and heavy metals [10,11,28].

Changes in pH were found to cause conformational transitions in human AFP, revealing a highly hydrophilic exposed molecular

surface at certain pH levels and extensive hydrophobic binding sites in crevices. The changes in AFP conformational structure and receptors occur in both acidic and alkaline pH media, exposing a large hydrophobic area on AFP. [29] Additionally, the treatment of homogenates with acid and alkaline pH resulted in structural modifications of receptors. Conformational changes of AFP molecules occur in at the alkaline pH of blood to protect the transported molecule, and in acidic intracellular pH conditions to release the transported molecule, such as a drug, inside the cell [10].

The binding of ^{125}I-AFP to cytosolic and nuclear receptors was found to be dependent on temperature and time, as shown in Figures 7 and 8. The maximum binding of ^{125}I-AFP to cytosolic receptors occurred at 45°C and 35°C after 5 hours for gastric adenocarcinoma and gastric lymphoma tissues, respectively. Meanwhile, the maximum binding of ^{125}I-AFP to nuclear receptors occurred after 7 hours at 35°C for gastric adenocarcinoma and after 4 hours at 45°C for gastric lymphoma tissues.

The reduction in the specific binding (SB%) after reaching the maximum binding at the optimum temperature and incubation time may be due to the denaturation of the tertiary structure of either the AFP receptors or ^{125}I-AFP, which would cause conformational changes. Alternatively, it may be the result of irreversible dissociation of the ^{125}I–AFP/AFP receptor complexes.

It was reported that changes in the external environment conditions of the reaction, such as temperature, can cause conformational changes of AFP molecules and their receptors [10]. According to Wu and Knight, the stability of AFP is dependent on its concentration and incubation temperature; when phosphate buffer or phosphate-buffered saline were used for specimen dilution, some AFP was lost [30].

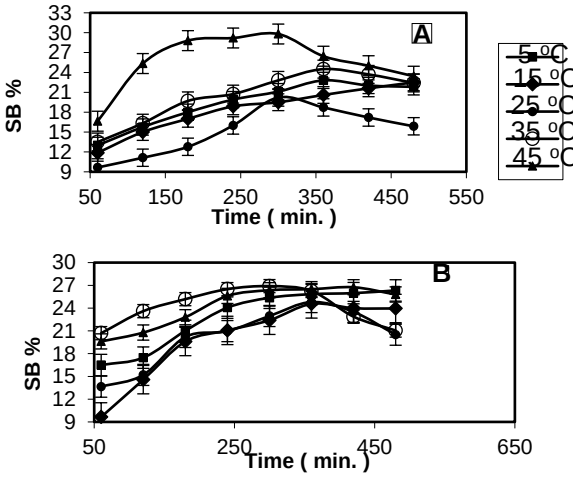

Figure 7. Time-temperature course of ^{125}I-AFP binding with its cytosolic receptors in: (A) gastric adenocarcinoma tissues and (B) gastric lymphoma tissues. The data points were plotted with a 5% error margin and a standard deviation of 1.0. The sample size was N=7 for gastric adenocarcinoma tissues and N=5 for gastric lymphoma tissues. The percentage of specific binding (SB%) was used to represent the affinity of ^{125}I-AFP with its cytosolic receptors. The results showed that the optimal temperature for cytosolic fractions of gastric adenocarcinoma and gastric lymphoma tissues were 45°C and 35°C, respectively, while the optimal time was 5 hours for both types of tissues. The Standard Errors Bars revealed a significant difference in the cytosolic receptors of gastric adenocarcinoma at 45°C, while there was no significant difference in gastric lymphoma tissues.

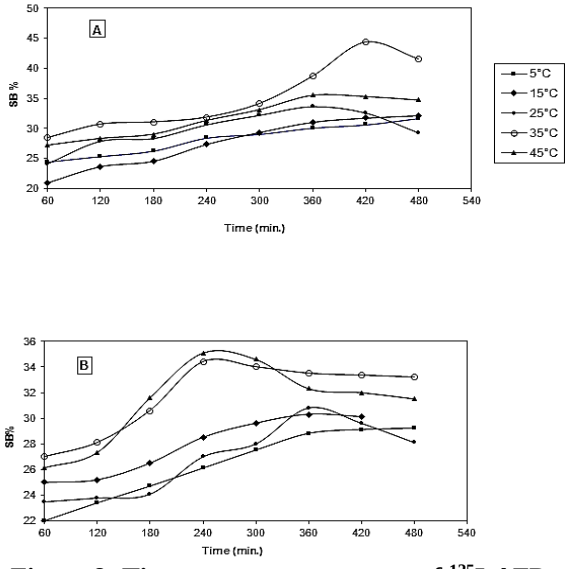

Figure 8: Time-temperature course of ^{125}I-AFP binding with its nuclear receptors in: (A) gastric adenocarcinoma tissues and (B) gastric lymphoma tissues. The data were graphed using a 5% error margin and a standard deviation of 1.0. The sample size was N=7 for gastric adenocarcinoma tissues and N=5

for gastric lymphoma tissues. The percentage of specific binding (SB%) was used to indicate the affinity of ^{125}I-AFP with its cytosolic receptors. The results showed that the optimal temperature for nuclear AFP receptors in gastric adenocarcinoma and gastric lymphoma tissues were 35°C and 45°C, respectively. Additionally, the optimal times were 7 hours and 4 hours for gastric adenocarcinoma and gastric lymphoma tissues, respectively. A significant difference was observed in nuclear receptors of gastric adenocarcinoma at 35°C and 7 hours, whereas there were no significant differences in gastric lymphoma tissues.

In comparison with our preliminary results, the specific binding percent values (SB%) increased with the optimal conditions of AFP receptor concentrations, 125I-AFP concentrations, and pH. The highest binding was achieved at the optimum temperature and time, resulting in a 26.8 percentage points (9.9-fold) and 25.4 percentage points (6.6-fold) increase for cytosolic AFP receptor binding and a 40.22 percentage points (10.8-fold) and 29.9 percentage points (6.8-fold) increase for nuclear AFP receptor binding in gastric adenocarcinoma and gastric lymphoma tissues, respectively.

In addition, our study found that the binding efficiency of AFP receptors with the 125I-AFP in nuclear fractions was higher than those in cytosolic fractions for both tissue types and the concentrations of AFP receptors in gastric adenocarcinoma were higher than those in gastric lymphoma tissues.

There has been an increased interest in studying AFP receptors due to their clinical and therapeutic applications. Some of these studies reported the existence of multiple AFP receptors on the cell membrane in the form of integral plasma transmembrane proteins [11]. The first group of AFP receptors was termed mucin receptors and was reported to be present on macrophages, monocytes, breast tumour cells, leukemic cells, and lymphoid cells [31]. The second type of AFP receptors was termed scavenger receptors. Researchers identified two scavenger receptors of 18 and 31 kDa on the lymphoid cell membrane that bound to AFP [6,32].

It was reported that there is a non-secreted form of AFP, termed cytoplasmic AFP (CyAFP) which can form in benign tumours and malignant cells, as well as in lymphoid-associated cells [5,11]. The cytoplasmic AFP form can bind with transcription factors and cytoplasmic nuclear receptors through a leucine zipper, as it contains a dimerization motif in the third domain polypeptide chain at the carboxyterminus [33].

4. Conclusion

A radioreceptor assay was performed to analyze ^{125}I-AFP binding with its receptors in both gastric adenocarcinoma and gastric lymphoma tissues and to investigate the concentrations of AFP receptors along with their binding properties in gastric cancer tissues at cellular and nuclear locations.

The preliminary tests to determine the concentrations of AFP receptors in cytosolic and nuclear fractions of the tissues were successful. Optimum conditions obtained for investigation of cytosolic AFP receptors in both tissue types were as follows: AFP receptor concentrations: 40.6μg/ml and 18.75μg/ml, respectively; 125I-AFP concentration: 14.28 x 10-10 M; pH: 8.0 and 7.8, respectively; temperature: 45°C and 35°C respectively; and time: 5 hr.

In addition, the optimal conditions obtained for quantitation of nuclear AFP receptors in gastric adenocarcinoma and gastric lymphoma tissues homogenates were as follows: AFP receptor concentrations: 25 μg/ml and 23.75μg/ml, respectively; 125I-AFP concentration: 14.28 x 10-10 M; pH: 7.6 and 7.4, respectively; temperature 35°C and 45°C, respectively; and time: 7 hr and 4 hr, respectively.

The specific binding percentage of 125I-AFP binding with its AFP receptor was found to increase under the optimum conditions and reached the maximum binding with an increment of 26.8 percentage points (9.9-fold) and 25.4 percentage points (6.6-fold) for cytosolic AFP receptors, and 40.22 percentage points (10.8-fold) and 29.9 percentage points (6.8-fold) for nuclear AFP receptors of gastric adenocarcinoma and gastric lymphoma tissues, respectively.

In addition, our study found that the binding efficiency of AFP receptors with the ^{125}I-AFP in nuclear fractions was higher than those in cytosolic fractions for both tissue types and the concentrations of AFP receptors in gastric adenocarcinoma were higher than those in gastric lymphoma tissues.

The development of the radioreceptor assay in this study presents a novel approach to detect AFP receptors and could be a recommended procedure for assessing cytosolic and nuclear AFP receptors in gastric adenocarcinoma and gastric lymphoma tissues in early cancer detection. This technique has the potential to aid the design of novel cancer detection and treatment approaches using AFP receptor-based nanoparticles and reveal novel mechanisms of antitumour immune response properties of AFP receptors in future studies.

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Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this manuscript.

Abbreviations

AFP, Alpha-fetoprotein; TB, Total binding; c.p.m., counts per minute; NSB, non-specific binding; SB, specific binding; SB%, percentage of specific binding; M, Molarity; hr, hour; min, minutes; µg, microgram; µl, microlitre; N, number of samples; rpm, revolutions per minute.

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