

Characteristics Studies of ^{125}I - anti total PSA antibody's Binding with prostate specific antigen (PSA) in Human Uterus Tumors.

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ABSTRACT

Two groups of uterus tumors (benign and malignant) postmenopausal patients were used to investigate the presence of prostate specific antigen (PSA). Preliminary experiments were performed to follow the binding of ^{125}I -anti total PSA antibody with PSA in uterus tissues homogenates of the two groups with their corresponding antigen and found to be (8.8, 7.1%) for benign and malignant tumors, respectively.

An ImmunoRadioMetric Assay (IRMA) procedure was developed for measuring PSA in benign and malignant uterus tumors homogenates. The optimum conditions of the binding of ^{125}I -anti total PSA antibody with PSA were as follows: PSA concentration (150, 200 μg protein), tracer antibody concentration (125, 250 μg protein), pH (7.6, 7.2), temp (15, 25°C) and time (1.5 hrs) for postmenopausal benign and malignant uterus tumors tissue homogenates, respectively.

The use of different concentrations of Na^+ and Mg^{2+} ions were shown to cause an increase in the binding at concentration of (125, 75 mM) of Na^+ ions and (75, 225 mM) of Mg^{2+} ions for benign and malignant uterus tumors homogenates, respectively, while the use of different concentrations of urea and polyethylene glycol (PEG) caused a decrease in the binding with the increase in the concentration of each of urea and PEG in the both cases.

INTRODUCTION

Prostate specific antigen (PSA) is a single chain glycoprotein that contain 7% carbohydrate⁽¹⁾. It is produced by epithelial cells lining the acini and ducts of prostate⁽²⁾. Functionally, PSA is a serine protease⁽³⁾ with trypsin and chymotrypsin-like activity^(4,5). A major portion of PSA exists in the circulation as a complex with α_1 -antichymotrypsin (PSA-ACT),

whereas a minor part exists as free form (f-PSA)⁽⁶⁾.

PSA was considered as a highly specific marker of prostate tissue⁽¹⁾ and the precursor forms have emerged as potentially important diagnostic serum markers for prostate cancer⁽⁷⁾. Also assessing PSA in children could be used as a potential marker in the diagnosis and follow-up of urogenital disorders⁽⁸⁾.

Many publications have confirmed that PSA is widely expressed at lower concentrations in prostate and in many non-prostatic tissues and fluids^(9,10) especially in

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female breast^(11,12). PSA was also expressed in salivary gland, pancreas and uterus⁽¹³⁾. Recently, an ectopic prostatic tissue in the uterine cervix was diagnosed in a 38-year-old woman, the glands were immunoreactive for prostatic specific antigen and prostatic acid phosphatase⁽¹⁴⁾.

The gene expression and protein production of PSA in these sources are under the regulation of steroid hormone via their receptors^(15,16). Furthermore, the expression of PSA is negatively regulated by P53, this provide a plausible explanation for a frequent increase of PSA levels in advanced prostate cancer⁽¹⁷⁾.

Several immunoassays are commercially available for measuring total serum PSA concentration including RadiImmunoAssay (RIA), ImmunoRadioMetricAssay (IRMA) using radioactive, enzyme, or fluorescent label⁽¹⁾. Recently, modified methods were used, for simultaneous measurement of (PSA- α_1 -ACT) complex together with free or total PSA⁽¹⁹⁾ and interleukin-6 (IL-6)⁽²⁰⁾.

The data of Iraqi registry center showed that there is a tendency toward an increase in the frequency of cervix and uterus cancer incidence during the last years⁽²¹⁾. Accordingly this study deals with modified of IRMA binding assay of total PSA in benign and malignant uterus tumors with ¹²⁵I-antitotal PSA antibody and the optimum binding conditions such as: reactants concentrations, pH, time, temp., monovalent and divalent salts in addition to the effect of urea and polyethylene glycol on this binding.

EXPERIMENTAL

Chemicals

All laboratory chemicals and reagents were of analar grade: Tris (hydroxy methyl) amino methane, Na-

K-tartrate, EDTA, and MgCl₂ were obtained from Fluka-Switzerland company, Dithiothreitol, polyethylene glycol, and NaCl from BDH-UK company, bovine serum albumin (BSA) from Sigma-USA company, Urea from May&Baker company, and total PSA kit purchased from Immunotech-Beckman Coulter Company-Czech Republic.

Apparatus

The apparatus used during this study were: Analytic balance, LKB gamma counter type 1270-rack gamma II, Pye unican pH meter, Cooling centrifuge type Hettichi, memmert incubator, memmert water bath, orbital shaker, and spectrophotometer ultra.

Patients:

Two groups of patients were included in this study, first group was involved 6 postmenopausal patients suffering from benign uterus tumors (age 55.7 years \pm 1.7) while the second group included 8 postmenopausal patients suffering from malignant uterus tumors (age 56.4 years \pm 2.3). The patients were clinically diagnosed by physicians and histologically proven by laboratory reports and were not taken any type of therapy.

All patients were admitted for treatment to Al-Habebia Hospital, Baghdad Educational Hospital, and Janeen Private Hospital, under the supervision of specialists: Dr.Hassan Fadhel, Dr.Kamel Jameel, Dr.Nada Al-Ebadie, and Dr.Huda Al-Alosie. They were histologically proven from the supervision of specialists: Dr.Nawal Alash, Dr.Luae Edward, and Dr.Awateff Al-Qurashee.

Collection of Specimens and Preparation of Tissue Homogenates

The tumor tissues were surgically removed from uterus tumor patients by hysterectomy. The specimens were cut off and stored immediately at -20°C prior to the study of the frozen tissue was pulverized on ice bath then homogenized at 4°C in TED buffer with a ratio of 1:3 (w:v) using a manual homogenizer. (TED buffer contains 0.01M tris(hydroxy methyl) amino methane, 0.15mM EDTA, and 1.2mM dithiothreitol). The homogenate was filtered through a nylon mesh sieve in order to eliminate filters of connective tissues then centrifuged at $1600 \times g$ for 20 min at 4°C . The supernatant was used as a source of PSA in this study.

METHODS

A-Estimation of Protein Contents:

Protein was determined by the method of Lowry *et.al.* (22) using bovine serum albumin as standard.

B-Preliminary Test of the Binding of PSA of Benign and Malignant Uterus Tumors Homogenates with ^{125}I -antitotal PSA antibody.

The binding of ^{125}I -antitotal PSA antibody with PSA in benign and malignant uterus tumors homogenates was primary measured according to the following:

1- Hundred micro liters of benign uterus tumors homogenate (592 μg protein) was incubated with $50\mu\text{L}$ of ^{125}I -antitotal PSA antibody (250 μg protein) at 25°C for 3.5 hrs with moderate horizontal shaking, the final volume was complete up to $500\mu\text{L}$ with TED buffer pH 7.4.

2- After incubation, the tube was centrifuged at $4000 \times g$ for 20 min to precipitate the formed complex (antigen-antibody). The supernatant was decanted, then the tubes were inverted on a filter paper for 10 min.

3- The amount of bound radioactivity (c.p.m) was counted in a gamma counter for 1 min.

4- Two additional tubes containing $50 \mu\text{L}$ of ^{125}I -antitotal PSA antibody only for total radioactivity were counted:

1- The experiment was repeated with malignant tumor homogenate (888 μg protein).

Calculations:

1- The radioactivity of each tube refers to the amount of PSA and represented by (B).

2- The (B/T%) was calculated where:

$$B/T\% = \frac{\text{Sample radioactivity (c.p.m)} \times 100}{\text{Total radioactivity (c.p.m)}}$$

C-Most Appropriate Conditions of Binding of Prostate Specific Antigen (PSA) of Benign and Malignant Uterus Tumors Homogenates with (^{125}I -Anti Total PSA Antibody):

Optimum Protein Concentration:

Fifty microliters of (^{125}I -antitotal PSA antibody) was incubated with $100\mu\text{L}$ of different amount of benign and malignant uterus tumors tissue homogenates (50, 100, 150, 200, and 250 μg protein). The final reaction volumes were complete up to $500\mu\text{L}$ with TED buffer pH 7.4. Then the step 2 to 4 of the section (B) was repeated.

Calculations:

1-(B/T%) was calculated as mentioned in section B.

2--(B/T%) was plotted against the corresponding protein concentrations for each case.

Optimum (^{125}I -Anti Total PSA Antibody) Concentration

The volumes of (25, 50, 75, 100, and 125 μl) of (^{125}I -Anti Total PSA Antibody) containing (125, 250, 375, 500, and 625 μg protein/reaction mixture), respectively were added each to 100 μl (150, 200 μg protein) of benign and malignant uterus tumors homogenates, respectively. The volumes were completed with TED buffer pH 7.4 to 500 μl . Then the steps 2 to 4 of the experiment B were repeated.

Calculations

1-(B/T%) was calculated as mentioned in section B.

2--(B/T%) was plotted against the corresponding (^{125}I -Anti Total PSA Antibody) concentration for each case.

Optimum pH

The optimum protein concentration was incubated with the optimum (^{125}I -Anti Total PSA Antibody) concentration in each case at 25°C for 3.5 hrs using TED buffer of different pH from 7.2 to 8.4. The final reaction volumes were 500 μl . Then steps 2 to 4 of the section B were repeated.

Calculations

1-(B/T%) was calculated as mentioned in section B.

2--(B/T%) was plotted against their corresponding pH values for each case.

Optimum Incubation Time

The optimum protein concentration was incubated with the optimum (^{125}I -Anti Total PSA

Antibody) concentration in each case at 25°C for several times intervals (1.5, 2.5, 3.5, 4.5, and 5.5 hrs) using the optimum assay buffer in each case. The final reaction volume was 500 μl . Then steps 2 to 4 of the section B were repeated.

Calculations

1-(B/T%) was calculated as mentioned in section B.

2--(B/T%) was plotted against their corresponding times for each case.

Optimum Temperature

The optimum protein concentration was incubated with the optimum (^{125}I -Anti Total PSA Antibody) concentration in each case at four different temperatures (15, 25, 37, and 45°C) for 1.5 hrs using the optimum assay buffer in each case. The final reaction volume was 500 μl . Then the step 3 and 4 of the section B were repeated.

Calculations

1-(B/T%) was calculated as mentioned in section B.

2--(B/T%) was plotted against their corresponding temperatures for each case.

D-Effects of different factors on the Binding of (^{125}I -Anti Total PSA Antibody) with PSA in Uterus Tumors Homogenates.

Effect of Ionic strength (exogenous Na^+ and Mg^{2+} ions):

The optimum protein concentration was incubated with the optimum (^{125}I -Anti Total PSA Antibody) concentration at optimum (incubation time and temperature to each case using the optimum assay

buffer for each case containing different concentrations of Na^+ and Mg^{2+} ions respectively (25, 75, 125, 175, and 225mM). The final reaction volume was 500 μl . Then steps 2 to 4 of the section B were repeated.

Calculations

1-(B/T%) was calculated as mentioned in section B.

2-(B/T%) was plotted against their corresponding Na^+ and Mg^{2+} ions concentration, respectively for each case.

Effect of Urea and Polyethylene glycol (PEG)

The pervious experiment was repeated by using the optimum assay buffer for each case contain different concentrations (3, 4, 5, and 6M) and (0.5, 1, 2, and 3%) of urea and PEG, respectively.

Calculations

1-(B/T%) was calculated as mentioned in section B.

2-(B/T%) was plotted against their corresponding Urea and PEG concentrations for each case.

RESULTS AND DISCUSSIONS

Preliminary Test of the Binding of PSA of Benign and Malignant Uterus Tumors Homogenates with ^{125}I -antitotal PSA antibody.

The prostate specific antigen (PSA) which was obtained from the homogenization of 14 cases of benign and malignant uterus tumors tissues reacts as an antigen when incubated with (^{125}I -Anti Total PSA Antibody) for 3.5 hrs then the bound PSA were measured by immunoradiometric assay (IRMA). Preliminary experimental conditions used resulted in (8.8 % and 7.1%) on postmenopausal benign and malignant uterus tumors patients, respectively. The enhancement of PSA

in uterus tumors patients may attribute to the raise in expression of PSA gene.

Several studies indicated that PSA should be considered as a (cancer fighter) at the tissue level and as available messenger indicator at the level of systematic circulation ⁽²³⁾. It has been thus suggested that effort to produce cancer vaccines on other therapies targeting PSA expression may be the wrong strategy and that treatment approaches to treat prostate and possibly breast cancer should be directed toward over expression of PSA at the tissue levels ^(24,25).

Most Appropriate Condition of Binding of Prostate Specific Antigen (PSA) of Benign and Malignant Uterus Tumors Homogenates with (^{125}I -Anti Total PSA Antibody):

The study of the binding of any antigen (e.g. hormone, drug, virus and etc) to its receptor necessitates the choice of the most appropriate conditions that lead to the maximum specific binding. Hence, the study of each of the following effect on the extent of the binding (^{125}I -Anti Total PSA Antibody) with PSA of benign and malignant uterus tumor tissues is quite necessary.

Optimum, Protein concentration

The alteration in the concentration of PSA of benign and malignant uterus tumor tissues influences the binding of this antigen to its specific antibody. To explore this fact practically, a set of increased concentrations of homogenate was prepared while the tracer and all other experiment conditions still fixed.

Figure (1) shows the quantitative precipitin curve in which the amounts of (Ab Ag) complexes that precipitate are plotted as a function of (Ag) concentration. The (Ab Ag) complex precipitate out of the solution because of the multivalent nature of both molecules. The

radioactive antibody (IgG) is directed against a single PSA molecule, and since the Ag IgG has two combining sites, it can crosslink antigenic sites of two different PSA molecules and from a lattice of interlocking molecules. As the size and complexing of the lattice increase, the lattice becomes insoluble and precipitate out of a solution ⁽²⁶⁾.

As shown in the same figure, when increasing concentrations of (Ag) were added the amount of the precipitate increased until a point of maximum binding was reached. After this point, as the amount of (Ag) increased the amount of precipitate diminished that may be due to the dissociation of the (Ag-Ab) complex

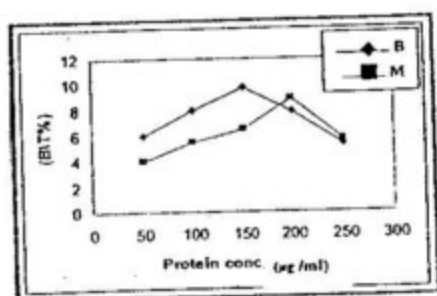


Fig.(1): The optimum concentration of PSA of benign and malignant uterus tumors in the reaction with ¹²⁵I-Anti Total PSA Antibody.

According, in all subsequent experiments 100 µl (150, 200, µg protein) for benign and malignant uterus tumor tissues were used respectively, since it gives highest binding.

Optimum (¹²⁵I-Anti Total PSA Antibody) Concentration

One of the most important criteria of the true Ag, is its saturability. To fulfil of this criterion and to estimate the suitable concentration of ¹²⁵I-Anti Total PSA Antibody, this experiment was carried out. The results are illustrated in figure (2) and show that the binding of ¹²⁵I-Anti Total PSA Antibody with PSA is

a saturable process but complete saturation however is theoretically never reached unless the amount of tracer used reach the saturation state ⁽²⁷⁾.

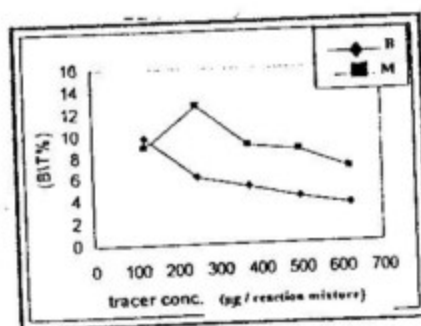


Fig.(2): The optimum concentration of ¹²⁵I-Anti Total PSA Antibody in the reaction with PSA of benign and malignant uterus tumors.

As shown in the same figure the PSA is used in the incubation mixture under the conditions of the experiments saturated with the tracer ¹²⁵I-Anti Total PSA Antibody when the concentration of the tracer was equivalent to 25 µl (125µg protein), 50 µl (250 µg protein) for benign and for malignant uterus tumors in a compate with previous studies on prostate cancer which indicated that the optimum tracer concentration were 80 µl (6400µg protein) ⁽²⁶⁾ and 0.36 mg/ml ⁽²⁸⁾. So in all subsequent experiments the above concentration of the tracer to each case was used.

Optimum pH

The effect of pH changes on the tendency of PSA of benign and malignant uterus tumor homogenates to associate with ¹²⁵I-Anti Total PSA Antibody was shown in figure (3).

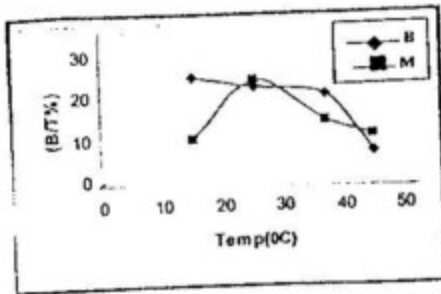


Fig.(3): The optimum pH for the binding PSA in benign and malignant uterus tumors with ¹²⁵I-Anti Total PSA Antibody.

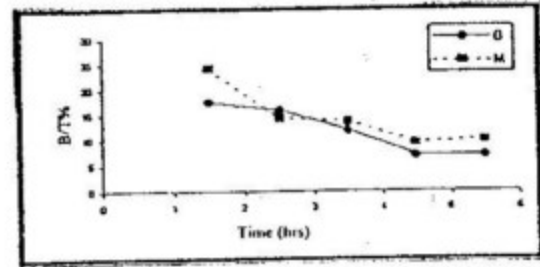


Fig.(4): The optimum incubation time for the binding PSA in benign and malignant uterus tumors with ¹²⁵I-Anti Total PSA Antibody.

This figure showed that the optimum pH were (7.6 and 7.2) for benign and malignant uterus tumors, respectively in comparable with other studies for prostate cancer 7.8⁽²⁶⁾, 7.2⁽²⁸⁾

These results indicated that the binding was pH dependent and the shift in the pH of the environment may affect the properties of the macromolecules involved in the binding. This effect includes the induction of protonation-deprotonation processes occurring with the ionizable groups of the amino acids present in the binding domain of the macromolecule⁽²⁹⁾, these results may also indicates that the immunoreactivity of the reactants or the stability of the formed complex enhanced at certain point of pH⁽³⁰⁾.

In view of these results, the buffers in all experiment were adjusted as above in each case.

Optimum Incubation Time

To choose the most appropriate incubation time, this experiment was carried out at different times intervals (1.5, 2.5,3.5, 4.5 and 5.5 hrs). Figure (4) represents the incubation time required to obtain maximum PSA binding with tracer.

The results indicated that when the incubation time increased the binding decreased in both of benign and malignant uterus tumors and the optimum incubation time was 1.5 hrs for each cases in comparable with other studies for prostate cancer (2 hrs)^(26,28). So in all subsequent experiment the incubation time was 1.5 hrs in each case.

Optimum Temperature

This investigation also includes the temperature depending of the binding between PSA in benign and malignant uterus tumor homogenates with ¹²⁵I-Anti Total PSA Antibody. Figure (5) show the effect of temperature on this binding.

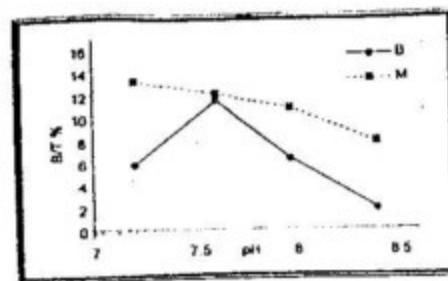


Fig (5): Optimum temperature for the binding PSA in benign and malignant uterus tumors with ¹²⁵I-Anti Total PSA Antibody.

The results indicated that the optimum temp. for the binding was (15 and 25 °C) for benign and malignant uterus tumors, respectively in

comparable with other studies in prostate cancer 4 °C ⁽²⁶⁾ and 45 °C ⁽²⁸⁾.

Effects of different factors on the Binding of (¹²⁵I-Anti Total PSA Antibody) with PSA in Uterus Tumors Homogenates.

Effect of Ionic strength (exogenous Na⁺ and Mg²⁺ ions):.

Figure (6) show the effect of Na⁺ and Mg²⁺ ions concentrations on the binding activity of PSA in benign and malignant uterus tumors with ¹²⁵I-Anti Total PSA Antibody.

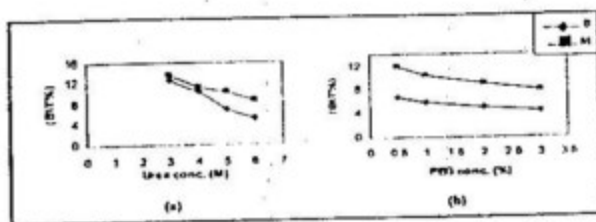


Fig (6): Influence of different concentrations of (a) Na⁺ ions (b) Mg²⁺ ions on the binding of PSA in benign and malignant uterus tumors with ¹²⁵I-Anti Total PSA Antibody.

The results of the fig (6-a) suggested that the highest binding will be found in the presence of (125 and 75 mM) of Na⁺ ion for benign and malignant uterus tumors, respectively. While fig (6-b) suggested that the highest binding will be found in the presence of (75 and 225 mM) of Mg²⁺ ion for benign and malignant uterus tumors, respectively.

These results may be due to the electrostatic interaction strength and the change in the ionic strength. Indeed if hydrophobic interactions were the force while stabilize (i.e.the metal ions may offer the nature of the hydrophobic forces necessary for the stabilization of biological membranes and affect the hydrophobic forces controlling the stabilization of the complex formed).

The interactions of these ions with the ionic groups of the (Ab-Ag) complex diminishes the (AbAg) interactions and therefore, increasing solubility of the complex, this effect can be explained according to the salting in phenomenon while when the solvent molecules were bound so tightly by the cations that they were unlike to solvate the (Ag-Ab) complex.Hence, the solute came of the solution (salting out phenomenon) .

Effect of Urea and Polyethylene glycol (PEG):-

Figure (7) summarize the effect of different concentrations of urea and polyethylene glycol (PEG) on the binding of ¹²⁵I-Anti Total PSA Antibody with PSA in benign and malignant uterus tumors homogenates.

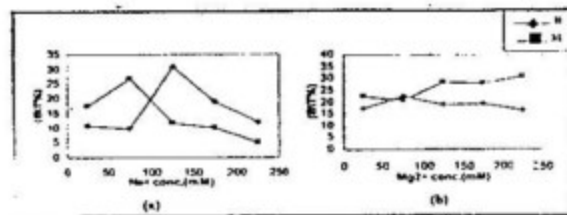


Fig (7): Influence of different concentrations of (a) Urea (b) PEG on the binding of PSA in benign and malignant uterus tumors with ¹²⁵I-Anti Total PSA Antibody.

Figure (7) indicates that the binding of PSA in benign and malignant uterus tumors with ¹²⁵I-Anti Total PSA Antibody decreased with increasing urea and PEG concentration, this effect can be attributed to the effect of urea on the hydrophobic forces between molecules. Since urea molecules complete for the hydrogen bonding sites and thus decreases the probability of formation the intramolecular hydrogen bonds between the R-groups ⁽³¹⁾

Also increasing concentration of PEG may result in precipitation of protein molecules which leads to decrease the interaction between PSA and ^{125}I -Anti Total PSA Antibody, and hence decrease the binding. This effect of PEG on the receptor protein solubility can be explained according to the steric exclusion mechanism ⁽³²⁾.

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دراسات توصيفية لارتباط الضد المعلم بنظير اليود المشع ذو العدد الكتلي 125 للمستضد النوعي البروستاتي الكلي بمستضدة في اورام الرحم

سامي المظفر ، جوان الصالحي

الخلاصة

تم التحري عن وجود المستضد الخاص بالبروستات (PSA) في مجموعتين من النساء باعمار ما بعد انقطاع الطمث مصابات بأورام الرحم الحميدة و الخبيثة. لقد دلت النتائج الاولية أن نسبة ارتباط الضد الموسوم بنظير اليود المشع 125 بالمستضد الخاص بالبروستات الموجود في مجانس أورام الرحم الحميدة و الخبيثة كان (7.1 و 8.8) بالتعاقب.

تم تطوير طريقة الفحص المناعي الأشعاعي لقياس المستضد الخاص بالبروستات في كل من أورام الرحم الحميدة و الخبيثة لتعيين الظروف المثلى لارتباط المستضد الخاص بالبروستات بضده الموسوم و كانت النتائج كما يلي: تركيز المستضد البروتيني الخاص بالبروستات (150 و 200 مايكروغرام / مللتر)، و تركيز الضد الموسوم (125 و 250 مايكروغرام / مزيج التفاعل) و الاس الهيدروجيني (7.6 و 7.2) و درجة الحرارة (15 و 25°م) اما الزمن فكان (1.5 ساعة) لكل من مجانس أورام الرحم الحميدة والخبيثة بالتعاقب و قد لوحظ من تجارب تأثير التراكيز المختلفة لايونات الصوديوم و المغنيسيوم زيادة الارتباط باستخدام التراكيز (25 او 75 ملي مولار) لايونات الصوديوم و (75 و 225 ملي مولار) لايونات المغنيسيوم في حالة اورام الرحم الحميدة والخبيثة بالتعاقب. بينما سبب استخدام تراكيز مختلفة لمادتي اليوريا و بولي اتلين كلايكلول انخفاض الارتباط مع زيادة التركيز لكل منهما في حالة أورام الرحم الحميدة والخبيثة.

Key words: Prostate specific antigen(PSA), Uterus tumors , ImmunoRadioMetricAssay (IRMA) , pH, Cations, urea and polyethylene glycol.