RESEARCH ARTICLE

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Fabrication of Antibody Conjugated Super Magnetic Oxide Nanoparticles for Early Detection of Prostate Cancer

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Abstract

Background: Prostate cancer is one of the most widespread cancers in the world. Early diagnosis is the most important factor in treatment efficiency. Furthermore, new methods for early diagnosis and treatment play an important role. In this study, we designed targeted conjugation of antibodies with iron nanoparticles and evaluated the binding properties of antibodies to prostate cancers and benign tissues. This method in addition to having a lower cost has high sensitivity and specificity. **Methods:** Anti- PSCA antibodies were purified and conjugated to super magnetic oxide nanoparticles (SPION). Then, iron staining on prostate adenocarcinoma tissues was performed. At the same time, immunohistochemically staining was performed on similar tissues to compare the results. In addition, benign prostatic hyperplasia (BPH) samples were used as a control sample. **Results:** In adenocarcinoma tissues with iron staining, many blue spots are seen compared to benign tissues, and the number of these spots increases with increasing tumor grade. **Conclusion:** These findings indicate the characteristic of iron staining as a conjugate antibody to iron can be an appropriate approach to specific staining of tumor markers in cancer tissues and can be used to diagnose prostate cancer due to its safety, low cost, sensitivity, and specificity.

Keywords: Anti-PSCA Antibody- SPION- Benign Prostatic Hyperplasia (BPH)- prostate cancer

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Introduction

Prostate cancer is one of the most common cancers in men over the age of 50 worldwide, and 1 in 6 American men has the disease (Archer et al., 2020). Despite the growing number of diagnosis methods, it remains one of the deadliest cancers. Common diagnostic methods and techniques used for this cancer are *in-vivo* and *in-vitro* (Zarghami et al., 1997; McClure et al., 2014). The development of the science of technology has provided the use of nanomaterials in the field of diagnosis and treatment of various cancers (Eatemadi et al., 2016; Mohammadian et al., 2016; Pahlavan et al., 2020). So in prostate cancer, both in the diagnosis and treatment, these nanomaterials are essential. These nanomaterials provide non-invasive therapeutic and diagnostic methods and do not damage healthy tissues (Kievit and Zhang, 2011; Davoudi et al., 2014; Nejati et al., 2021). Among the different types of nanomaterials, superparamagnetic iron oxide particles have received more attention due to their unique properties and wide range of applications in biomedical (Mahmoudi et al., 2011). These nanoparticles have an iron oxide core that can be used for specific purposes through its external magnet. SPIONs have special features such as superparamagnetic, high irreversibility, high structural field, extra anisotropy contributions, or shifted loops after field cooling (Kodama, 1999). On the other hand, the degradability, low toxicity, and small size of these materials have made them easily usable in the human body (Bartolozzi et al., 1999; Tartaj and Serna, 2003; Meng et al., 2009). Despite the many benefits of these materials, their use alone may reduce their effectiveness because their binding in the body may be very weak in the target cells. Given that one of the characteristics of these materials is their ability to bind to different types of biomolecules, antibodies, peptides, and aptamers (Koneracká et al.,

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1999; Wang et al., 2008; Agemy et al., 2010). In a study by Agemy et al., (2010) nanoparticle-bound CREKA pentapeptide was used to treat and image blood vessels in prostate cancer. Therefore, by binding these biomolecules, the efficiency of these materials can be easily improved (Mehta et al., 1997; Koneracká et al., 1999; Koneracka et al., 2002). This binding is possible through both the carboxyl group and the amin group (Montenegro et al., 2013; Farahani et al., 2019). Because tumor cells in each tissue have their own antigens and antibodies against them that conjugate to nanoparticles, can easily bind to these antigens. As a result, the intended purpose, which may be diagnosis or treatment, can be done more accurately. PSCA is a 123 amino acid protein belonging to the Thy-1 / Ly-6 family that attaches to the cell surface via a GPI anchor (Reiter et al., 1998). This antigen was first detected in prostate cancer and mRNA PSCA is expressed in more than 80% of these cancers, but its expression is very limited in normal prostate tissue (Gu et al., 2000). On the other hand, overexpression of PSCA protein is associated with Gleason degree and pathological stage and androgen-independent status (Gu et al., 2000). So in benign prostatic hyperplasia (BPH) and low-grade PIN, its expression is low and most expression is seen in HGPIN type (Zhigang and Wenlv, 2004). PSCA protein expression is up-regulated in metastatic castration-resistant prostate cancer (Raff et al., 2009). Therefore, it is an important marker for diagnostic and therapeutic purposes in this cancer. Due to the cellular surface of this protein, it is easy to use an antibody against it to detect this antigen and conjugate this antibody with nanoparticles and improve the diagnostic methods of this cancer (Reiter et al., 1998; Kievit and Zhang, 2011). Immunohistochemistry is one of the antigen-antibody-based diagnostic methods that is used in prostate cancer to diagnose different degrees of cancer (Leite et al., 2010; Duraiyan et al., 2012), But in addition to its advantages, this method also has disadvantages that are expensive and due to the toxicity of the substrate DAB, can be harmful in the long run (Hofman, 2002). As the male population ages and the mortality rate from other causes decreases, concern for early detection and effective treatment of prostate cancer will grow, so we have to look for diagnostic methods that are both cheap and have high sensitivity and specificity and at the same time are safe. Accordingly, in this study, For the first time, we used iron staining with anti-PSCA antibody conjugated to SPION nanoparticles to early diagnose prostate cancer.

Materials and Methods

Purification of Anti-PSCA Polyclonal Antibody

In this study, we used antibodies prepared in previous studies by (Chizari, M., et al) (Chizari et al., 2021). Before purifying the antibody using a chromatographic column, in order to better purify the antibody against PSCA, first all IgGs in the serum were precipitated using saturated ammonium sulfate. For this purpose, 7.6 g of ammonium sulfate was weighed and dissolved in 10 ml of distilled water on heat until it reached close to boiling temperature, then kept in a closed container overnight for one day. In the next step, the serum of the immunized rabbits was centrifuged for 10 minutes at 10,000 g, and after the formation of sediment, the supernatant was transferred to a new tube. Then, add 450 µl of the saturated ammonium sulfate supernatant (in contact with solid crystals) drop by drop and mix. Finally, a milky-colored precipitate formed. The microtubes were centrifuged at 10,000 g for 20 minutes, then, the supernatant solution was isolated and the precipitate was dissolved in 1 ml of PBS. Before starting the purification process, we determined their concentration by the nanodrop device's absorbance reading at 280 nm.

Purification of Antibodies Using Affinity Chromatography

To purify anti-PSCA antibodies, N- hydroxysuccinimide (NHS) -activated agarose (Pierce Chemicals Co., IL, and the USA) was used (Gong et al., 2016; Faramarzi and Dolatabadi, 2017). The amount of 2 mg of PSCA peptide was weighed and dissolved in 3 ml of coupling/wash buffer. Add a bottle of active agarose to the column. Allow settling at room temperature, then rinse with distilled water and then coupling/wash buffer. After adding the prepared peptide to the column and placing it on a shaker for 2 hours at room temperature after time the solution of the inside of the column was removed and washed with 1ml of coupling/wash buffer. At this stage, add 1ml of binding/wash buffer and after the solution comes out, add 2ml of rabbit serum containing antibodies along with 2ml of binding/wash to the column and put it at room temperature for 2 hours. Next, wash the column with 5ml of binding/wash buffer and add 8ml of elution buffer (1.1 M Glycine. HCL with PH = 2.5-3) to release the PSCA antibody. Then add a neutralizing buffer (1MTris with pH = 9). We increased its pH from 7.2 to 7.4 and measured the amount of fractured protein by absorption at 280 nm with a nanodrop device.

Detection of Rabbit Antibody against PSCA by ELISA

To ensure purified PSCA protein, ELISA was performed (Farajollahi et al., 2012) by dissolving 1 µg of BSA-conjugated peptide per well in phosphate buffer, capping the bottom of the plate, and incubating overnight at 4°C. In the next step, the plate was washed 3 times with phosphate buffer (PBS 1% and Tween 20 0.01% in dH_2O), Phosphate buffer containing 5% skim milk powder, and 0.05% tween with pH = 7.4 was prepared as a blocking buffer. Three hundred microliters were poured into each ELISA wells and incubated for 1.5 to 2 hours at room temperature. After washing with phosphate buffer, different dilutions of antibodies were prepared (1/100- 1/12,800) and 100 µl was coated in the plate well and incubated for 1 hour. After washing three times, 100 microliters of Goat anti-rabbit (IgG-HRP Santa Cruz Biotechnology, CA, USA) were incubated for 30 minutes with a dilution of 1.5,000 (Lin, 2015). After washing, 100 microliters of tetramethylbenzidine liquid substrate (TMB, Roche) were added and after observing the deep blue color, 100 microliters of 0.2 M sulfuric acid were added and then the light absorption of each well was read at a wavelength (OD) of 450 nm by the ELIZA reader (Model 680, Bio-Rad, CA, USA).

Preparation of Hydrazide-Activated SPIONs

Spions with dextran coating (dexSPIONs) containing COOH group (nanomag®-CLD-spio 100 nm, Micro-mod Partikel technologies GmbH, Rostock, Germany) with 20 nm diameter with adipic acid dihydrazide (Sigma Aldrich, USA), were functionalized. For this purpose, we dissolved 8 mg of adipic acid in 5 ml of 0.1 M sodium phosphate with pH = 6.0. Dissolve 2.5 mg of 20 nm nanoparticles in the above solution. Then 8 mg of water-soluble carbodiimide 1-Ethyl-3- (3-dimethyl aminopropyl) carbodiimide (EDC) (Thermo Fisher, USA) was added to the mixture and completely dissolved by stirring figure 1. Allowing the reaction to stand for 4 hours at room temperature. Dialyze this solution overnight to remove reaction by-products and additives components in the PBS buffer containing 0.1 M sodium chloride (Hermanson, 2013).

Activation of Antibodies with Sodium Periodate

For activation of antibodies 0.5mg/ml of the antibody was mixed with 10 µl of periodontal sodium solution in the dark to dissolve and react for 15 to 20 minutes. After this time, immediately stop the reaction by adding 25 micrograms of sodium sulfite (Na2SO3). The oxidized antibodies were then immediately purified using a filtration gel column (by dissolving Sephadex G50) and the antibody concentration was measured using a nanodrop at 280 nm (Hermanson, 2013). We mixed 0.5 mg/ml of the antibody with a concentration of 0.863 mg/ml with 500 µl of nanoparticles with a concentration of 5 mg/ml. The nanoparticle was bonded to the antibody for 2 hours at room temperature. By adding 50 µl of 1 M ethanolamine with pH = 9.6, the unreacted aldehyde groups were blocked. This was done in 30 minutes. After this step to remove salts and other excess compounds, the conjugated antibody was dialyzed overnight in a 0.01 M sodium phosphate buffer containing 0.15 M sodium chloride. Then the conjugated antibody was stored at 4°C. We used the MACS column to evaluate the conjugation result (Xu et al., 2011).

Sample preparation

In this project, 77 paraffin samples of prostate adenocarcinoma in different grades in duplicate and 50 paraffin samples of benign prostatic hyperplasia as duplicates were prepared from Shahid Hashemi Nejad Hospital in Tehran. Some of these samples were selected from 2019 and some from 2021. The consent form for these patients (IR.TBZMED.REC. 1,400.644) was completed and then all the characteristics of the samples including sampling method, PSA and FPSA level, slide grade, invasion of surrounding tissues, and age were completely recorded. All slides were examined by a pathologist for confirmation of sampling accuracy and grade of slides. Due to the fact that in this project we have two types of staining, a complete set of adenocarcinoma slides and benign prostatic hyperplasia was isolated for immunohistochemical staining and another for iron staining.

Immunohistochemical staining

A staining kit (Uromab mouse –rabbit micro polymer detects IHC DDK Italy) from the (baft ara gene) company was purchased for immunohistochemical staining. Briefly, all benign and adenocarcinoma prostate sections were deparaffinized at 60°C for 20 minutes and cleared in xylene. Then rehydrated through immersion in reducing grades of alcohol. Subsequently, methanol containing 0.3 % hydrogen peroxide (H2O2) was used to suppress the activity of endogenous peroxidase. After washing the tissue sections three times in Tris Buffered Saline (TBS), the slides were autoclaved for 10 min in sodium citrate buffer (pH= 6.0) to retrieve the antigen. The slides were treated overnight at 4C with the following antibody dilutions as the optimal dilution for subsequent use: anti-PSCA antibody using a 1/20 dilution. The next day slides were washed with TBS. Polymer enhancer was added for 10 min, and then incubated with the secondary antibody, En VisionTM +/HRP Dua1Link Rabbit/Mouse (Dako. Denmark) for 1 h at room temperature. Subsequently, visualization of the antigen was achieved through 3, 3' -diaminobenzidine (DAB) chromogen substrate for 10 min at room temperature followed by counterstaining with hematoxylin (Dako). Finally, sections were dehydrated with graded alcohol, cleared in xylene (Dako), and mounted for evaluation.

Iron staining

At this stage, after melting the paraffin slides and rehydration, we placed the slides in the EDTA-Tris pH = 9 buffer for 10 minutes in an autoclave at 126 degrees. The primary antibody conjugated to the nanoparticles of SPIONs was added to the slides and placed overnight at 4°C. After washing with Tris buffer, we painted samples by Prussian Blue Iron Stain Kit (Polysciences, USA) and after washing, Nuclear Fast Red (Polysciences) was used for 5 minutes. Then after the washing and dehydration stage, sections are mounted for evaluation. Then the intensity of color in relation to the grade of slides was checked and scored by a pathologist.

Statistical Analysis

Pearson's χ^2 method was used for the clinical association of PSCA expression with either immunohistochemical staining or iron staining. The Mann-Whitney method was used to compare the expression intensity of PSCA with immunohistochemical and iron staining in cancer and BPH groups as well as ELISA results. P values less than 0.05 were considered significant.

Results

Evaluation of purified antibodies

The purified specific antibody was diluted to a dilution of 1/12,800 and the results of indirect ELISA showed a purified antibody titer greater than 1/1,600. In this test, non-immune rabbit serum was used as a negative control, and immune serum produced by Chizari et al., (2021) was used as a positive control Figure 2. With increasing serum dilution, light absorption decreased and there was a significant difference between purified antibodies and

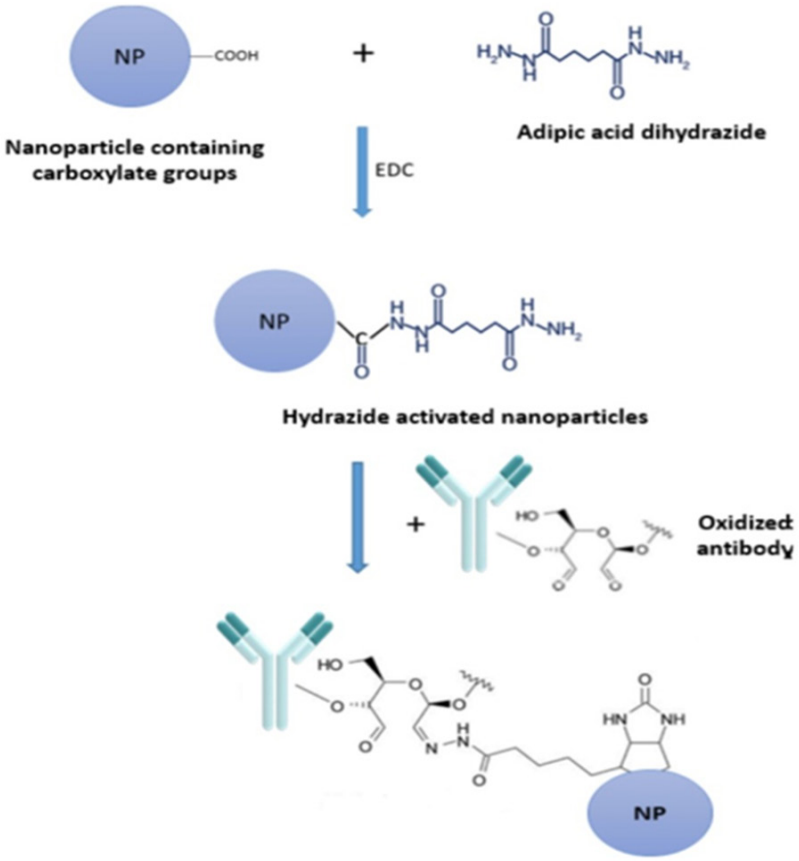

Figure 1. Scheme of Bio-Conjugation for anti-PSCA Antibody-dexSPIONs. DexSPIONs were functionalized by adipic acid dihydrazide via EDC. Carbohydrate positions of antibodies were oxidized using sodium periodate and linked toward activated dexSPIONs.

normal rabbit serum IgG (negative control) up to 1,600 dilution. (p<0.0001).

Evaluation of PSCA antigen expression pattern in prostate tissues (adenocarcinoma, benign hyperplasia) by immunohistochemistry and iron using Anti-PSCA-Ab

To evaluate and compare the pattern of iron staining with immunohistochemical staining in the epithelial cells of prostate glands, paraffin blocks of adenocarcinoma and BPH were used and immunohistochemistry and iron tests were performed on the tissues using purified antibodies. As shown in Table 1 and Figure 3, an increase in PSCA marker expression was observed in prostate cancer tissues compared to BPH or benign hyperplasia by the IHC method (P <0.0001). In line with IHC results, PSC marker expression was increased in iron staining compared to BPH (P <0.0001).

Evaluation and analysis of PSCA expression pattern in adenocarcinoma, BPH tissues using Anti-PSCA-Ab

The study population in this project was 77 prostate cancer tissues and 50 BPH tissues. The degree of tumor malignancy in patients was classified by Gleason score (Gs). The degree of the tumor indicates the different degrees of malignancy as follows:

Gs; 6: Well differentiated,
Gs; 7(3+4): Moderately differentiated,
Gs; 7 (4+3): Moderately poorly differentiated,
Gs; 8: Poorly differentiated &
Gs; 9-10: Undifferentiated tumors

Of the 77 prostate cancer tissues, 6 had Gs; 6 (9.1%), Gs; 7 (3 + 4) 28 (36.4%), Gs; 7 (4 + 3) 12 (15.6%),

Table 1. Comparison of PSCA Expression in Adenocarcinoma and BPH by Immunohistochemical Staining and Iron Staining. PSCA marker over expression was observed in prostate cancer tissues compared to BPH by the IHC method (P <0.0001)

Intensity of staining	PCa (IHC-staining) No. (%)	BPH (IHC-staining) No (%)	PCa (Iron-staining) No. (%)	BPH (Iron-staining) No (%)	PSCA-IHC expression (p value; Pearson χ2)	PSCA-Iron expression (p value; Pearson χ2)
No reaction	0	30 (60)	13(16.9)	30 (60)		
Weak	33(42.9)	20 (40)	26(33.8)	18 (36)		
Moderate	29(37.7)	0	33(42.9)	2 (4)	<0.0001	<0.0001
Strong	15(19.5)	0	5(6.5)	0		
No.total	77	50	77	50		

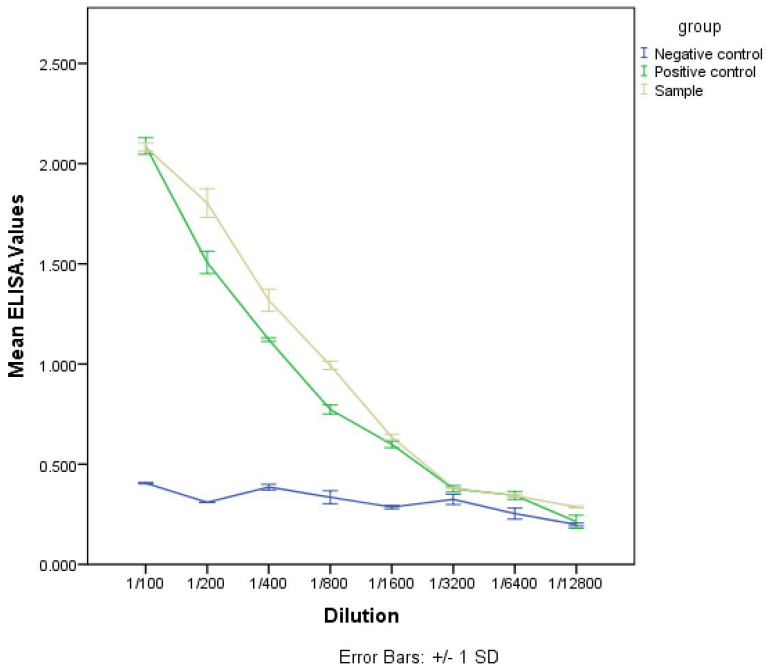

Figure 2. The Diagram above Shows the Adsorption Read in the Titers of 1/100 to 1/2800 of the Purified Antibody. By increasing serum dilution, light absorption decreased and there was a significant difference between purified antibodies and normal rabbit serum IgG (negative control) up to 1600 dilution. ($p<0.0001$)

Gs; 8 had 17 (22.1%) and Gs; 9-10 13 (16.9%). Based on the results of Table 2, a significant relationship was observed between Gleason degree and IHC (P = 0.005) as well as iron staining (P = 0.007), so that with increasing Gleason degree, the intensity of IHC and iron staining increased. Significance was found between tumor invasion to perineural and staining intensity (P = 0.051).

Discussion

The spread of science and increasing advances in this field have created a new world in the diagnosis and treatment of many diseases and cancers, especially nanoparticles, which are one of the most fundamental advances (Salmani Javan et al., 2022). Nanoparticles due to their unique properties including optical (Mahmoudi et al., 2010), electronic (Poizot et al., 2000), polymeric (Alagheband et al., 2022; Nejati et al., 2022), and magnetic properties (Tari et al., 1979) are more noticeable. In one study, an anti-PSCA monoclonal antibody, human 7F5 mAb bound to Fe_3O_4 / gold nanoparticles (Gold Mag) was used as a PSCA-specific molecular MRI probe to diagnose prostate cancer in the body (Meinel et al., 2012). Among the types of nanoparticles, SPIONs are the most widely used in medicine. These nanoparticles are of an iron oxide core that can be used for specific purposes through its external magnet (Mahmoudi et al., 2011). Gao et al., (2012) was used dual-purpose nanoparticles that

Table 2. Patient Classification based on Some Clinical Parameters Including Gleason Degree and Tumor Spread to Surrounding Areas. Also, their relationship with PSCA expression intensity by immunohistochemical and iron staining. By increasing Gleason degree, the intensity of IHC and iron staining increased. Significance was found between tumor invasion to perineural and staining intensity (P = 0.051)

Patients and tumor characteristics	No (%)	PSCA expression-IHC staining (p value; Pearson $\chi 2$)	PSCA expression-Iron staining (p value; Pearson $\chi 2$)
Gleason scores;			
Gs 6	7(9.7)	0.005	0.007
Gs7(3+4)	28(36.4)		
Gs7(4+3)	12(15.6)		
Gs8	17(22.1)		
Gs9-10	13(16.9)		
Extraprostatic extension			
Positive	6(7.8)	0.8	0.313
Negative	71(92.2)		
Perineural invasion			
Positive	42(54.5)	0.051	0.124
Negative	35(45.5)		

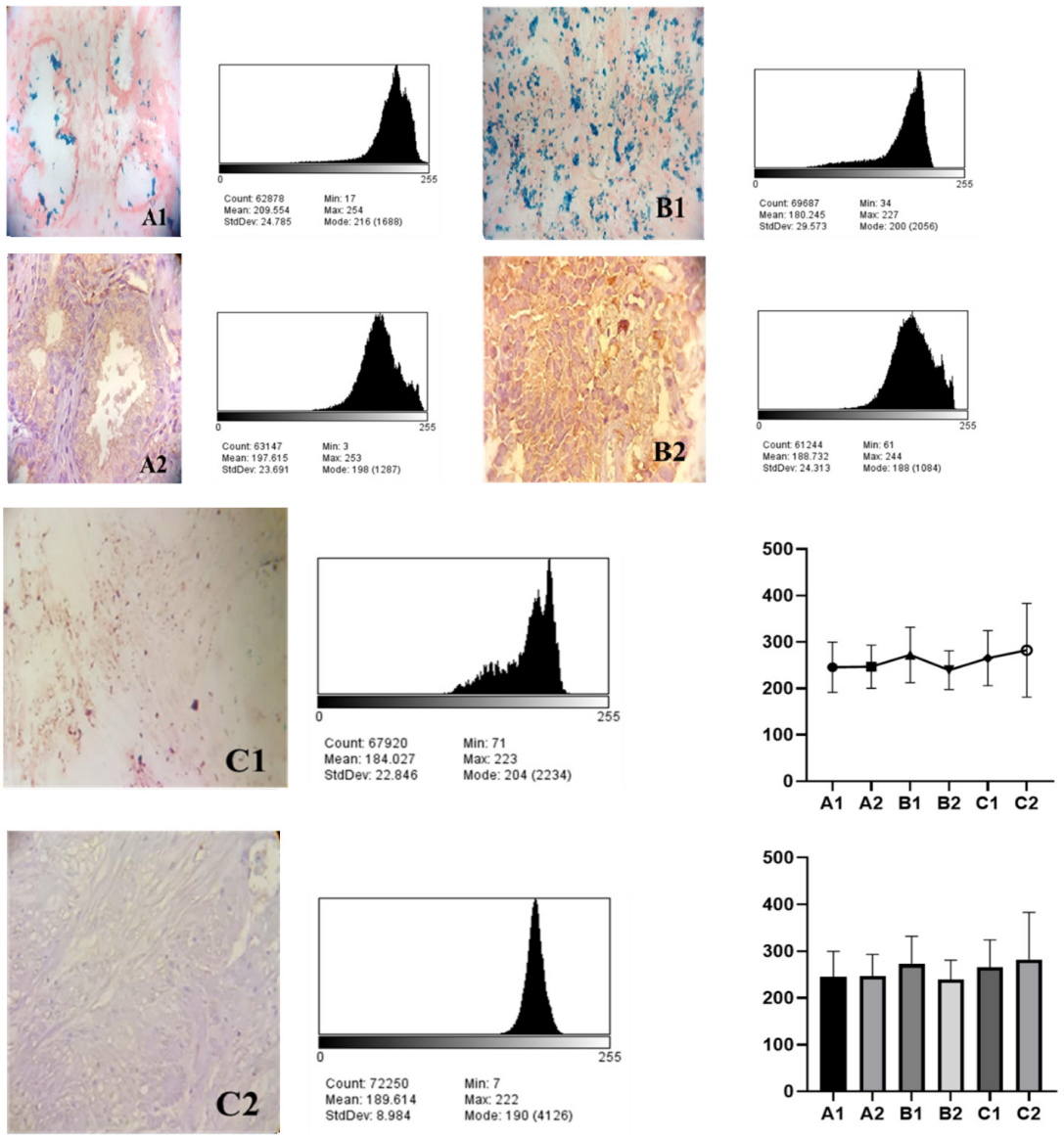

Figure 3. Iron Staining Pattern and Immunohistochemistry of Prostate Cancer Tissues (A1, A2, B1, B2, C1, and C2) and BPH. PSCA marker expression was incresead in prostate cancer tissues compared to BPH or benign hyperplasia by the IHC method (P <0.0001). In line with IHC results, PSC marker expression was increased in iron staining compared to BPH (P <0.0001). A1: Prostate cancer tissue with glycine grade 3 + 4, which is stained with iron; A2: Same tissue with immunohistochemical staining; B1: Gleason grade 5+4 prostate cancer tissue with iron staining; B2: Same tissue with immunohistochemical staining; C1: Benign prostatic hyperplasia tissue with iron staining; C2: The same tissue with immunohistochemical staining.

have anti-PSCA antibody single chains and are attached to the surface of SPION nanoparticles *in vivo*. A drug release pattern of more than 764 hours was observed. In this project, we used superparamagnetic iron oxide nanoparticles (SPIONs) for iron staining of prostate cancer tissue specimens. For this purpose, in the first phase of the project, we selected the PSCA antigen, which is a cell surface antigen that increases in prostate cancer as glayson levels increase (Reiter et al., 1998; Gu et al., 2000) and its expression pattern is different in normal and cancer cells and therefore, it is used as an effective biomarker in the diagnosis, treatment, and prognosis of prostate cancer (Yang et al., 2014). We purified the anti-polyclonal antibody produced by Chizari et al., (2021) using NHS activated agarose column and then the antibody titer was measured by ELISA. ELISA results showed high titers of this antibody. By concentrating the polyclonal antibody and determining the concentration by the nanodrop device, the antibody was prepared for the second phase. In the second phase, conjugation of antibodies was performed with iron nanoparticles. In a study by Taeb et al., (2014) antibodies against PSCA peptides were conjugated to iron oxide superparamagnetic nanoparticles by sulfo SMCC method. Although the results of measuring the concentration of protein and iron in the conjugate solution showed successful conjugation, the final results of iron staining were poor. One possible reason for this failure could be a decrease in antibody affinity for the antigen due to non-targeted conjugation to the nanoparticle. Therefore, in this study, targeted conjugation method was

used. The chemical adipic acid dihydrazide was used to activate dexSPIONs. The carboxyl group of the antibody was then targeted to functionalize the antibody with dexSPIONs. In a study by Farahani et al., (2019), both carboxyl and amino groups were used to functionalize antibodies, and a comparison of the results showed that the signal from conjugation through the carboxyl group was more intense than the amine group. Finally, the design for the third phase was prepared by creating (anti-PSCA antibody-dexSPIONs). By preparing adenocarcinoma and benign prostatic hyperplasia slides in duplicate from paraffin blocks in the pathology laboratory of Shahid Hasheminejad Hospital and examining the slides by the pathologist in terms of grade and accuracy of the prepared samples, the slides for immunohistochemical staining and iron staining were prepared. In this study, the binding properties of antibodies to prostate cancer and benign tissues were evaluated by immunohistochemical staining using PSCA-specific antibodies and iron staining using conjugated PSCA-specific antibodies to iron particles with SPION. Immunohistochemical staining is a powerful tool for detecting advanced cancer and uses primary and secondary antibodies to detect antigens (Leite et al., 2010). PSCA expression increased in patients with prostate cancer compared with BPH by IHC analysis. Also in adenocarcinoma tissues, PSCA expression increases with increasing malignancy. IHC analysis in this study also indicated that a direct relationship was found between the degree of malignancy or geleason score and the intensity of PSCA expression in prostate cancer tissues. In other words, with increasing intensity of PSCA marker staining, the degree of malignancy increases. The results of IHC staining on PC3 prostate cancer cells by Taeb et al., (2014), also showed that the intensity of staining increased with increasing Gleason degree. Despite the advantages of this diagnostic method, the disadvantages of this (Duraiyan et al., 2012) include: its high cost, which is mainly related to its primary and secondary antibodies and the other hand, the use of DAB as a substrate for secondary conjugated antibodies to the enzyme has toxic effects and creates problems for the expert in the long run, led us to look for an alternative diagnostic method that is both relatively inexpensive and has high specificity and sensitivity. For this purpose, we used optimized iron staining and first treated the conjugated polyclonal antibodies (anti-PSCA antibody-dexSPIONs) with adenocarcinoma and BPH slides with exactly the same grade as we used for IHC, so the antibody tissue surface was ligated to PSCA antigen. Then iron staining was performed and the results showed that the intensity of the dye increased with increasing tumor grade and many blue dots were observed in high-grade slides compared to BPH slides and this is due to increased PSCA antigen. In fact, the pattern of antigen expression of the PSCA gene in the iron staining method is the same as the IHC staining. In 2019, this staining method was performed by Farahani et al., (2019) on PC3 prostate cancer cells and their studies showed that the oriented conjugation method promoted the efficiency of targeting tumor antigens, and the presence of iron particles might enhance MRI image intensity in vivo by targeting PSCA-overexpressing cells in future studies. In the present study, by performing this staining method on prostate cancer tissues, we obtained specific staining from conjugated antibodies to nanoparticles in the tissue.

In conclusion, in this project, in line with immunohistochemical results, tissue-specific staining with specific PSCA antibodies conjugated to iron nanoparticles indicated that the intensity of iron staining increased with increasing malignancy or Gleason degree. These findings indicate the characteristic of iron staining as a conjugate antibody to iron and can be a good alternative to specific staining of tumor markers in cancer tissues.

Abbreviations and acronyms

BPH: Benign prostate hyperplasia

GS: Gleason scor

HGPIN: High-Grade prostatic interaepithelial neoplasia

IHC: Immuno histochemistry

NHS-Activated agarose: N-hydroxysuccinimide activated agarose

PAP: Prostate acide phosphatase

PSA: Prostate specific antigen

PSCA: Prostate stem cell antigen

PSMA: Prostate specific membrane antigen

SPION: Super paramagnetic iron oxide nanoparticle

Author Contribution Statement

Writing - original draft preparation: Tina Nayerpour dizaj and Davoud Jafari-Gharabaghlou; critical review of study: Mahdi Farhoudi Sefidan Jadid and Rana Jahanban; Scientific advisor: Mandana Rahimi, Mohammad Farajollahi and Conceptualization, Supervision: Nosratollah Zarghami and Monireh Mohsenzadegan.

Acknowledgements

Not applicable.

Availability of supporting data

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Consent to participate

The consent form for the patients (IR.TBZMED.REC. 1400.644) was completed.

Ethical Approval and Consent to participate

This research protocol was evaluated and approved by Tabriz university of medical sciences research committee (IR.TBZMED.REC. 1400.644).

Consent to publication

Informed consent was obtained from all individual participants included in the study.

Compliance with Ethical Standards:

All procedures performed in studies involving

human participants were in accordance with the ethical standards of tabriz university of medical sciences research committee (IR.TBZMED.REC. 1400.644) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Competing Interests

No potential competing interest was reported by the authors.

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