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Original Article

Quantification of Human Chorionic Gonadotropin by Bovine Serum Albumin Nanoparticles

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A B S T R A C T

Background and Aims: Some nanoparticles can be used in immunoassays to increase sensitivity. This study aimed to evaluate a novel nano-immunoassay based on bovine serum albumin nanoparticles (BSA NPs).

Materials and methods: At first, the nanostructure was synthesized, and then applied as a tag in the nano-immunoassay. Then the concentration of β-subunit of human chorionic gonadotropin (βHCG) in the clinical samples was quantified by traditional enzyme-linked immunosorbent assay (ELISA), and then checked by the nano-immunoassay.

Results: The Pearson's correlation coefficient between ELISA and nano-immunoassay was high, i.e., 0.80. Relative sensitivity and specificity of this nano-immunoassay were reported 97.4% and 96.6%, respectively.

Conclusions: BSA NPs can be applied in nano-immunoassays as a new structure, and as an example, βHCG can be detected by this novel assay.

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Introduction

To detect antigens, different immunoassay methods have been established, which are based on the interaction between antigen (Ag) and antibody (Ab) [1]. Enzyme immunoassay, radioimmunoassay, fluorescence immunoassay, and chemiluminescence immunoassay can be mentioned as some examples [2-4].

Nowadays, nanoparticles have brought many great advantages and new opportunities in different areas, including chemistry, physics, biology, and medicine. Moreover, they can be used for detection of molecules. They are also recognized as good candidates for labeling of antibodies, due to unique chemical and optical properties [5]. For instance, various nanoparticles, e.g. gold nanoparticles can be used in immunoassays [5]. Interestingly, the use of nanoparticles can give us higher signals, compared with the traditional tags [6]. Hirch et al. showed that the aggregation of antibodies and nano-shell conjugates are ideal to monitor the antibody [7]. Moreover, Puertas et al. demonstrated the efficacy of a nano-immunoassay based on magnetic nanoparticles (MNPs) [8]. Moreover, silver nanoparticles (Ag NPs) were proved to be a good bio-label for electro-analytical immunoassay [9]. In another study, Zhou et al. reported the use of three-dimensional plasmonic nano-antenna-dots array to enhance the immunoassay's fluorescence and detection sensitivity [10]. Bovine serum albumin nanoparticles (BSA NPs) are recognized as important structures which can be used for detection, imaging, and treatment purposes [9].

In the present study, a new structure is introduced for immunoassay, based on BSA NPs, and compared with common immunoassay tags. In fact, BSA NPs were synthesized, and used to detect β subunit of human chorionic gonadotropin (βHCG).

Materials and Methods

This lab trial study was conducted on some clinical samples. BSA, silver nitrate ($AgNo_3$), and nicotinamide adenine dinucleotide (NADH) were provided from Sigma-Aldrich Company (St Louis, USA). The enzyme-linked immunosorbent assay (ELISA) plates, ELISA kits, and βHCG standards were sourced from DiaPlus Inc., Canada.

Synthesis and characterization of BSA nanoparticles

The precipitation method was used to synthesize BSA NPs. In the first step, 25 mL of ethyl alcohol (70% v/v) and 100 µL of formaldehyde (10% v/v) were gently added to 25 mL of BSA at concentration of 500 mg/mL, and shaken at 200 rpm for 20 minutes at room temperature. In the next step, synthesized BSA NPs were centrifuged at 3000 g for 5 minutes, and washed three times by distilled water (DW). The concentration of BSA NPs was adjusted to 500 µg/mL by DW, and BSA NPs were characterized by scanning electron microscopy (SEM) (Hitachi, S-2400, Japan).

Preparation of samples

Totally 522 serum samples were obtained from different medical laboratories in Yazd, Iran.

Five mL of venous blood were obtained from women aged 20-50 years with no smoking habits. The blood samples were placed in the propylene plastic tubes, incubated at room temperature for 30 minutes, and centrifuged at 850 g for 15 minutes. In the next step, the serum of each blood sample was isolated and stored at -70 ºC until measurement of βHCG. All women signed an informed consent form.

Evaluation of nano-immunoassay

The serum concentration of βHCG in all samples was firstly checked by ELISA kit, and then by our nano-immunoassay. For ELISA, according to the kit instructions, 100 µL of each serum sample was added to ELISA microplate wells previously coated with βHCG antibody, incubated for 30 minutes at 37 °C, and washed three times by the washing buffer. Then, 100 µL of secondary antibody conjugated with horseradish peroxidase were added, incubated at 37 °C for 30 minutes, and rinsed three times with washing buffer. Next, 50 µL of substrate (H_2O_2) and 50 µL of tetramethylbenzidine were added, incubated at 37 °C for 15 minutes, and stopped with 50 µL of 1N HCl. Ultimately, the optical density (OD) of each well was read at 450 nm by ELISA reader (ELICO, UK), and βHCG concentration of samples was measured, according to the standard curve, which was previously obtained by serial concentrations of known βHCG solutions.

In the case of nano-immunoassay, similar to ELISA, 100 µL of each serum sample were added to antibody-coated wells, incubated for 15 minutes at 37 °C, and washed three times with washing buffer. These wells were the same as ELISA wells. Then, 100 µL of BSA NPs were added to each well, incubated for 15 minutes at 37 °C, and again rinsed three times with washing buffer. Next, 100 µL of $AgNo_3$ (100 mg/mL) and 100 µL of NADH (50 mmol/L) were added, and incubated 15 minutes at room temperature. Then, the OD of each well was read at 340 nm and divided by the optical absorbance of negative control. Finally, βHCG concentration of each sample was separately calculated, according to the standard curve. To obtain the standard curve, serial concentrations of βHCG solution were used instead of clinical samples. In the case of negative control, βHCG solution was not used, and normal saline was applied. Fig. 1 is the schematic demonstration of the nano-immunoassay.

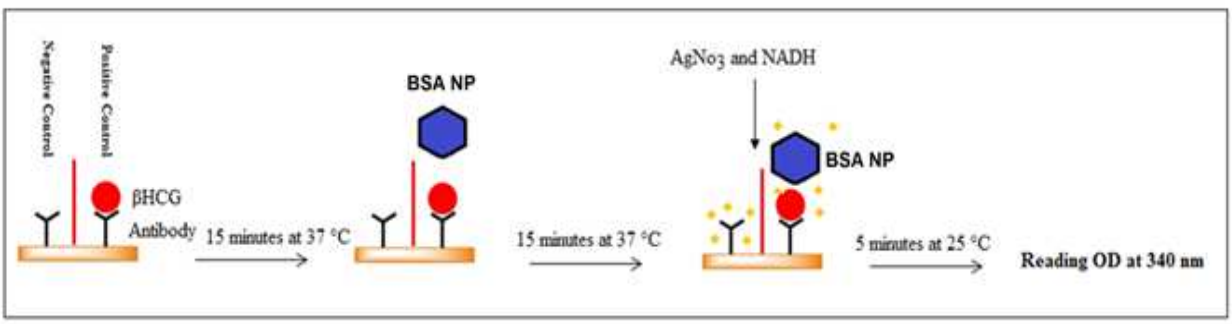

Fig. 1. The schematic representation of the novel nano-immunoassay

Statistical analysis

The study results are shown as the mean ± standard deviation (SD) with three replicates. Pearson's correlation coefficient was calculated to obtain the correlation between the ELISA and the nano-immunoassay techniques.

Results

Characterization

The SEM image of BSA NPs is shown in Fig. 2. As it can be observed, their shape was approximately spherical, and the size range was near 30-50 nm. Their zeta potential was +35.5 mV (at pH = 7 and ionic strength of 50 mM).

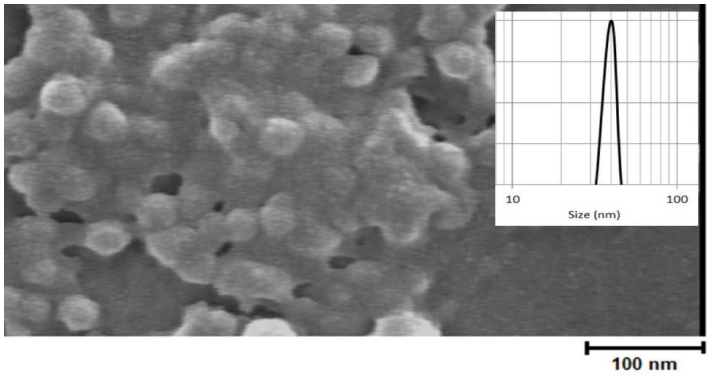

Fig.2. The SEM image of BSA NPs.

Evaluation of Nano-immunoassay

The correlation between concentration of βHCG and $OD_{test}/OD_{control}$ for nano-immunoassay is demonstrated in the Fig. 3a. As it is seen, its R^2 was 0.85. The correlation between ELISA and nano-immunoassay is demonstrated in the Fig. 3b. As indicated, R^2 for immunoassay was 0.80.

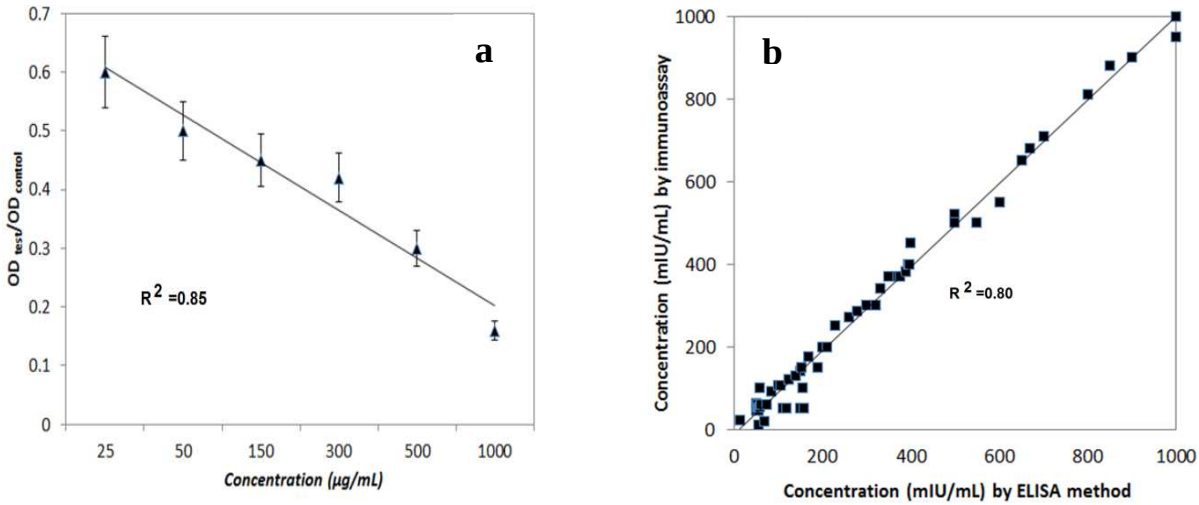

Fig. 3. The correlation between concentration of βHCG and $OD_{test}/OD_{control}$ (a). The correlation between ELISA and nano-immunoassay (b). The results are shown as Mean ± SD with n=10 for each concentration.

Diagnostic sensitivity, diagnostic specificity, predictive value of positive (PVP), predictive value of negative (PVN), false discovery rate (FDR), and false positive rate (FPR) are demonstrated in Table 1. Moreover, reproducibility and the percentage of relative standard deviation (RSD) within and between days are demonstrated in Table 2.

Table 1. All parameters related to the nano-immunoassay, compared with ELISA

Sample Size	522
True Positive (TP)	303
False Positive (FP)	8
True Negative (TN)	200
False Negative (FN)	11
Diagnostic Sensitivity =TP/TP+FN	97.4
Diagnostic Specificity =TN/FP+TN	96.6
Predictive Value of Positive =TP/TP+FP	97.7
Predictive Value of Negative =TN/FN+TN	96.2
False Discovery Rate =FP/TP+FP	0.022
False Positive Rate =FP/FP+TN	0.033

Table 2. The reproducibility (mean, standard deviation and relative standard deviation) values of the nano-immunoassay for 3 concentrations (50, 150 and 500 mIU/mL) of βHCG

	Mean ± SD	RSD (%)
Within Days, N=20		
1	50 ± 0.7	1.4
2	150 ± 2.5	1.7
3	500 ± 15	3
Between Days, N=20		
1	50 ± 0.7	1.4
2	150 ± 2.7	1.8
3	500 ± 16	3.2

Discussion

The aim of the present study was to evaluate a novel nano-immunoassay, based on BSA NPs. It is proposed that the difference in ion absorption between negative control and positive samples can give us good signals regarding quantification of analytes. Although this study revealed a good correlation between concentration of βHCG and $OD_{test}/OD_{control}$

with a good linearity, improvement of the method must be taken into consideration in the future studies. The results obtained from clinical samples indicated good sensitivity, specificity, reproducibility, PVP, and PVN, as well as low FDR and FPR. It must be noted that the number of clinical samples is an important factor in these studies, and can affect all the parameters. This factor needs to be addressed further in the future studies. In the present study, ELISA was used as the gold standard method, because of its low cost, rapid testing protocol and its common usage in medical laboratories. However, other methods must also be compared with these nano-based methods in future. In the current study, the new protocols were used for quantification of serum βHCG, since it is regarded as a good protein model, but variable results may be achieved for other analytes. As mentioned in the materials and methods section, NADH was applied after absorption of nitrate ions. NADH can reduce Ag ions, and synthesize Ag nanoparticles. This is to say that the end products of this reaction are NAD^+ and Ag nanoparticles. Hence, the decrease in OD was observed at 340 nm, and the increase in OD was reported at 420 nm because Ag nanoparticles have a sharp peak at 420 nm. Although nitrate ions have absorption at 340 nm, their intensity was not adequate for immunoassay, that NADH can increase its intensity. Since no data have been published in literature regarding the use of BSA NPs for detection and quantification, the present study can be the first study of its kind. Of course, there are some studies which have used nanoparticles for immunoassay, among which Hirsch et al. described a rapid nano-based immunoassay to detect analytes without any sample preparation. They spectroscopically monitored the aggregation of antibody and nano-shell conjugates in the presence of analyte [7]. Puertas et al. designed a novel nano-immunoassay based on the magnetic nanoparticles (MNPs), and mentioned that the application of functionalized MNPs improved the sensitivity of lateral-flow immunoassay [8]. In another study, Porter et al. used Ag NPs as a bio-label for electro-analytical immunoassay, and found that Ag NPs could lead to electro-analytical amplification when attached to the analyte (approx. 10^6) [9]. Zhou et al. reported the use of three-dimensional plasmonic nano-antenna-dots array to enhance the immunoassay's fluorescence and detection sensitivity [10]. Moreover, Huo et al. stated a facile nanoparticle immunoassay using gold nanoparticles (Au NPs) coupled with dynamic light scattering method to discover cancer biomarkers. In their study, serum samples were mixed with a citrate-protected Au NP solution, and then antibody solution was added. Finally, the difference in size of Au NPs was detected by dynamic light scattering [11]. In most of the studies, researchers have used optical properties of various nanoparticles, though chemical property of BSA NPs was not applied. Based on the findings of previous studies, there are different electrochemical immunosensors or enzyme biosensors in regard with detection of βHCG [12-14]. Taken together, the BSA NPs have nitrate adsorption property, which can be used in immunoassay together with antibody or aptamer. The authors

suggest that other analytes also need to be examined via these immunoassay methods.

Conclusion

It can be concluded that BSA NPs may be a good tag in nano-immunoassay to detect βHCG. The results of the nano-immunoassay have a good correlation with ELISA results. It is worth mentioning that this nano-immunoassay must be further evaluated.

Conflict of Interest

No conflict of interest is addressed.

Acknowledgments

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