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CASE REPORT

Pseudo-Elevation of Anti Double Stranded DNA IgG Antibody Caused by Rheumatoid Factor

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SUMMARY

Background: **The examination of anti-double stranded DNA (ds-DNA) IgG antibody is of great significance for the diagnosis, differential diagnosis, assessment of disease activity, and prognosis of disease recurrence in SLE.**
Methods: **We used a chemiluminescence method to detect ds-DNA IgG and found that the levels of ds-DNA IgG antibody in the patient's serum were significantly increased and the indirect immunofluorescence (IIF) test result was negative. Laboratory tests show that the patient's RF level far exceeds the upper limit of their reference range.**
Results: **RF 110.6 IU/mL, ds-DNA IgG 753 IU/mL; After PEG6000 treatment, the RF was 108.7 IU/mL, and then the ds-DNA IgG was measured at 23.5 IU/mL.**
Conclusions: **The RF IgM subtype is the main cause of RF interference in IgG antibody detection, mainly due to the binding of the Fc region of RF to the Fab segment of IgG. Combining with capture antibodies and labeled antibodies leads to the formation of non-specific detection signals, or directly reacting with the detected substance, resulting in false positive test results.**
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KEYWORDS

ds-DNA antibody IgG, pseudo-elevation, interference, rheumatoid factor

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory connective tissue disease that plays an important role in clinical research [1]. It can be found in multiple organs, and the number of early, mild, and atypical cases is rapidly increasing [2]. The laboratory examination of SLE, especially the detection of anti-ds-DNA IgG antibody is of great significance for the diagnosis, differential diagnosis, assessment of disease activity, and prognosis of disease recurrence in clinical SLE [3,4]. Among various autoimmune antibodies, only anti-ds-DNA IgG antibody is almost exclusively present in the serum of SLE patients, serving as serological markers and specific antibodies for SLE. The changes in the titer of anti-ds-DNA IgG antibody are correlated

with the activity of SLE [5,6]. Anti-ds-DNA IgG antibody testing is the most commonly performed method in clinical practice for screening autoimmune diseases, especially for testing SLE diseases. Therefore, anti-ds-DNA IgG anti-body was chosen as the detection indicator for this experiment.

Rheumatoid factor (RF) is a pathological globulin that is mainly divided into three types: IgM, IgG, and IgA [7]. It can bind to denatured IgG or IgG in human and animal immune complexes. RF can interfere with the detection of thyroglobulin, herpes simplex virus IgM antibody, novel coronavirus IgM antibody, and tumor markers. However, there have been no reports on whether RF interferes with the detection of anti-double-stranded DNA antibodies. We investigated the interference of RF against ds-DNA IgG antibody in the serum of a patient with abnormally elevated anti-ds-DNA IgG antibody who was IgG antibody positive but had negative clinical symptoms.

CASE PRESENTATION

The patient is a 36-year-old female with urgency and frequency of urination for 4 months and worsening for 5 days. She was admitted with a urinary system infection. The patient had right side low back pain 5 days ago with fever and hematuria. Since the onset of the disease, the patient has suffered from sore throat and no history of hypertension, heart disease, and diabetes. Physical examination shows no joint swelling or pain, and no rash. Laboratory tests for anti-nuclear antibodies, anti-neutrophil cytoplasmic antibodies, and indirect immunofluorescence are negative. RF 110.6 IU/mL (reference range 0 - 15 IU/mL), ds-DNA IgG 753 IU/mL (reference range 0 - 30 IU/mL); After PEG6000 treatment, the RF was 108.7 IU/mL, and then the ds-DNA IgG was measured at 23.5 IU/mL.

DISCUSSION

In this study, we used Yahuilong (Shenzhen, China) chemiluminescence method to detect ds-DNA IgG and found that the levels of ds-DNA IgG antibody in the patient's serum were significantly increased, but the patient did not have corresponding clinical symptoms, and the indirect immunofluorescence (IIF) test result was negative. Therefore, we suspect that the patient's ds-DNA IgG antibody levels are falsely elevated. Laboratory tests show that the patient's RF level far exceeds the upper limit of their reference range. The results are shown in Table 1.

RF is one of the common interfering substances in the immune response, and the polyclonal RF IgM subtype is the main reason for RF interfering with IgG antibody detection. RF cannot bind to all IgGs, and it mainly binds to the Fc region of RF and the Fab segment of IgG. It can interfere with the capture or labeling of antibodies in the detection system, forming steric hindrance and causing false negative results. It can also combine with captured antibodies or labeled antibodies to form non-specific detection signals, or directly react with the tested substance, resulting in false positive test results. The amount of RF interference generated by the body is not clear. Both high and low levels of RF can interfere with the immune response. RF can bind to IgG in fetal bovine serum to form an immune complex, and the Fc segment of the immune complex can bind to RF again to achieve the blocking effect, as shown Figure 1.

Research has shown that RF has the characteristics of anti IgG antibodies, which can react with the FC segment of IgG in humans and animals, making the impact of RF on immunological detection increasingly apparent, leading to biased detection results. It should be noted that the three main subtypes of RF in serum, IgM, IgG, and IgA, require further research on their interference mechanisms, interference thresholds, and interference effects. Among them, IgA-RF and IgM-RF are relatively easy to detect, but the detection of IgG type RF is more difficult. About half of IgG type RF will be missed, so it is also an important component of "hidden rheumatoid factor" [8].

The reason for the generation of RF is likely due to the long-term invasion of viruses, mycoplasma, and other substances. This persistent infection stimulates the body, causing changes in the immune system, triggering an immune response, producing antibody IgG, which denatures one's own IgG. The denatured IgG is then converted into a new antigen, prompting the body to produce anti denatured IgG antibodies (i.e., anti-antibodies), which are rheumatoid factors. RF is released in the form of follicles in peripheral blood, lymph nodes, and tonsils, which can promote the normal operation of the immune system and activate complement and clear immune complexes [9,10]. Due to RF interference in immunoassay, it has the characteristic of binding to the Fc segment of immunoglobulin IgG. Binding to antibodies in the reagent can cause false positives or false negatives in the test results.

PEG6000 can precipitate different types of immunoglobulins, so whether RF interferes with detection in the sample cannot be predicted in advance [11]. When patients exhibit abnormal results that do not match clinical symptoms and their RF levels increase, we have reason to suspect the existence of interference. We can choose the PEG6000 precipitation method based on RF subtypes to reduce false positives and provide more accurate experimental evidence for clinical practice. Furthermore, due to the advantages of low cost, simple operation, and good interference removal effect of using PEG6000 to remove RF, it is recommended for routine clinical testing.

Declaration of Interest:
All authors declare that they have no conflict.

Table 1. RF and dsDNA IgG values before and after PEG6000 treatment.

NON	PEG6000
RF (IU/mL)	
110.6	108.7
ds-DNA IgG (IU/mL)	
753	23.5

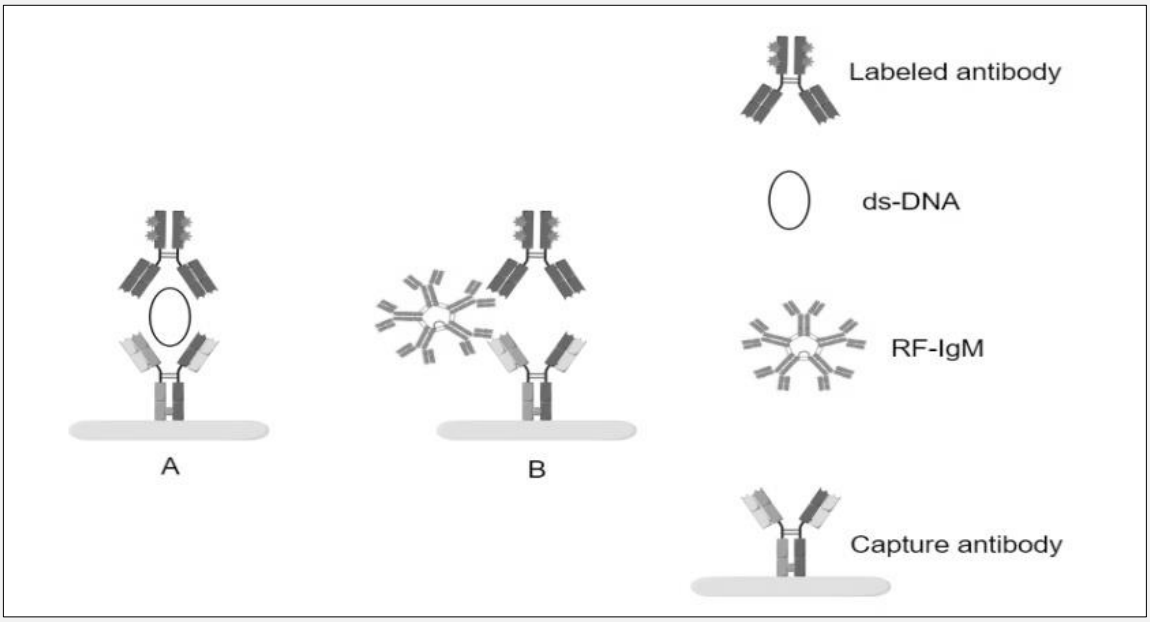

Figure 1. ds-DNA IgG antibody IgG antibody detection interference mechanism diagram.

A. ds-DNA IgG antibody IgG antibody schematic diagram of normal detection mechanism. B. ds-DNA IgG antibody IgG antibody schematic diagram of false positive mechanism.

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