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The detection of IgG class antibodies against SARS-CoV-2 nucleocapsid protein by application of nanoparticles

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Abstract. – OBJECTIVE: Enzyme-linked immunosorbent assay (ELISA) is a widely used biochemical analytical method for the detection of a biomarkers, through a specific antigen-antibody reaction. A common with ELISA is the amount of concrete biomarker falling below the detection limit. Thus, the approach that will contribute to enhanced sensitivity of enzyme-linked immunosorbent assay is of great importance for medical practice. To address this issue, we used nanoparticles to improve the detection limit of traditional ELISA.

MATERIALS AND METHODS: 80 samples were used, for which the presence of IgG antibodies against SARS-CoV-2 nucleocapsid protein were already determined qualitatively. We tested the samples using an *in vitro* ELISA kit [SARS-CoV-2 IgG ELISA, COVG0949 (NovaTec, Leinfelden-Echterdingen, Germany)]. Additionally, we tested the same sample with the same ELISA kit but with the addition of 50 nm diameter citrate-capped silver nanoparticles. The reaction was performed, and data were calculated according to manufacturer guidelines. To measure ELISA results absorbance (optical density - OD) at 450 nm was read.

RESULTS: Greater absorbance values have been revealed in case of silver nanoparticles application (66 cases, 82.5%, $p<0.05$). ELISA with application of nanoparticles classified 19 equivocal cases as positive and 3 equivocal ones as negative, 1 negative case as equivocal.

CONCLUSIONS: Our findings suggest that nanoparticles can be used to improve the sensitivity of ELISA method and increase the detection limit. Thus, it is logical and desirable to enhance the sensitivity of ELISA method by application of nanoparticles; the approach is low cost and with a positive impact on accuracy.

Key Words:
Nanoparticles, ELISA, IgG.

Introduction

Enzyme-linked immunosorbent assay (ELISA) is a widely used biochemical analytical method for detection of a target substance through a specific antigen-antibody reaction. The method is a highly specific, simple, stable, and rapid tool for biomarkers detection[1]. Biomarkers[2,3], which are biological substances, can be measured and objectively evaluated as indicators of concrete processes at cellular, tissue, organ, or whole-body level. Advances in cell biology and genetics, molecular pathology and precision medicine facilitated the use and importance of biomarkers for clinical trials, analytic epidemiology, and disease management[4,5]. The amount of concrete biomarker in biological sample is frequently below the detection limit of ELISA method. Thus, the approach that will contribute to enhanced sensitivity of enzyme-linked immunosorbent assay is of great importance for medical practice.

To address this issue, we used nanoparticles to improve the detection limit of traditional ELISA. Specifically, we tested whether 50 nm citrate capped silver nanoparticles could improve ELISA performance for quantitative determination of immunoglobulin G (IgG) class antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleocapsid protein.

Materials and Methods

All samples were analyzed using both, a standard ELISA method and ELISA method modified by the addition of nanoparticles. The ELISA kit was the same in both sets [NovaLisa® SARS-CoV-2 (COVID-19)] IgG ELISA for *in vitro* diagnostic use [Product number – COVG0949 (NovaTec Immunodiagnostica GmbH, Germany)]. The nanoparticles were 50 nm diameter citrate capped silver nanoparticles (AGCB50-1M, Bio-Pure Silver Nanoparticles – bare citrate, nanoComposix, San Diego, CA, USA).

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The reaction was performed, and data were calculated according to manufacturer guidelines. To measure ELISA results absorbance (optical density - OD) at 450 nm was read to determine the selection and application of the nanoparticles. In each vial of ELISA microplate were added 5 ml of nanoparticles stock solution [mass concentration 1.0 mg/mL, atomic (Ag) molarity 9.27 mmol/L, molarity particle concentration – 1.5×10^{12} particles/mL, Ag mass percent (%) – 0.1, max optical density (cm^{-1}) – 120].

The results were calculated according to manufacturer's guidelines: sample absorbance value x 10 / cut-off control absorbance value (NovaTec Units – NTU). The results were interpreted as following: (a) positive (> 11); (b) equivocal (9-11); and (c) negative (< 9).

Statistical Analysis

The results were analyzed statistically by application of SPSS v.27 software (IBM Corp., Armonk, NY, USA) for the aim to determine their statistical significance, $p<0.05$.

Results

The data of ELISA analysis with (OD-Ag and NTU-Ag) and without (OD and NTU) 50 nm

Table I. Results of ELISA analysis with (OD-Ag and NTU-Ag) and without (OD and NTU) 50 nm diameter citrate capped silver nanoparticles application.

#	OD	OD-Ag	NTU	NTU-Ag	#	OD	OD-Ag	NTU	NTU-Ag
1	2.747	2.800	27.55	28.1	41	0.607	0.600	6.09	6.02
2	0.736	0.816	7.38	8.19	42	2.322	2.400	23.29	24.07
3	2.416	2.440	24.23	24.47	43	2.3	2.390	23.1	23.97
4	1.77	1.800	17.75	18.05	44	1.032	1.144	10.35	11.47
5	1.243	1.344	12.47	13.48	45	1.485	1.502	14.89	15.07
6	0.922	0.822	9.25	8.24	46	1.84	1.867	18.46	18.73
7	0.97	0.833	9.73	8.36	47	2.392	2.402	23.99	24.09
8	2.735	2.844	27.43	28.52	48	1.155	1.167	11.58	11.71
9	2.721	2.766	27.29	27.74	49	2.143	2.244	21.49	22.51
10	1.9	1.967	19.06	19.73	50	0.978	1.018	9.81	10.21
11	1.282	1.484	12.86	14.88	51	2.468	2.500	27.75	25.08
12	1.018	1.110	10.21	11.13	52	1.027	1.098	10.3	11.01
13	1.094	1.222	10.97	12.26	53	0.817	0.820	8.19	8.22
14	1.041	1.107	10.44	11.10	54	2.04	1.978	20.46	19.84
15	0.947	0.989	9.44	9.92	55	0.953	1.098	9.56	11.01
16	0.841	0.870	8.44	8.73	56	0.911	0.901	9.14	9.04
17	1.645	1.700	16.5	17.05	57	0.888	0.800	8.91	8.02
18	0.995	1.190	9.98	11.94	58	1.924	2.010	19.3	20.16
19	2.681	2.688	26.89	26.96	59	0.918	1.099	9.21	11.02
20	0.98	0.920	9.83	9.23	60	2.414	2.449	24.21	24.56
21	2.535	2.744	25.43	27.52	61	2.467	2.400	24.74	24.07
22	0.944	0.887	9.47	8.90	62	2.524	2.544	25.32	25.52
23	2.714	2.835	27.22	28.44	63	2.558	2.601	25.66	26.09
24	0.971	1.134	9.74	11.37	64	0.889	0.900	8.92	9.03
25	1.696	1.954	17.01	19.60	65	1.795	1.865	18	18.77
26	1.08	1.334	10.83	13.38	66	2.691	2.544	27	25.52
27	1.009	1.588	10.12	15.93	67	0.899	0.990	9.02	9.93
28	1.056	1.277	10.59	12.81	68	2.462	2.503	24.7	25.11
29	0.991	1.102	9.94	11.05	69	0.976	1.099	9.6	11.02
30	0.849	0.800	8.52	8.02	70	1.185	2.001	11.86	20.07
31	1.418	1.633	14.22	16.38	71	2.207	2.211	22.14	22.18
32	0.81	0.755	8.12	7.57	72	2.768	2.800	27.76	28.08
33	1.108	1.133	11.11	11.36	73	1.185	1.180	11.89	11.84
34	2.32	2.114	23.27	21.20	74	0.852	0.850	8.55	8.53
35	1.119	1.178	11.22	11.82	75	0.894	0.849	8.97	8.52
36	0.992	1.117	9.95	11.20	76	1.796	1.801	18.01	18.06
37	2.032	2.200	20.38	22.07	77	1.288	1.304	12.92	13.08
38	1.025	1.111	10.28	11.14	78	2.181	2.190	21.88	21.97
39	2.673	2.600	26.81	26.08	79	1.574	1.605	15.79	16.10
40	1.148	1.202	11.51	12.06	80	0.998	1.112	10.01	11.15

Table II. Statistical parameters calculated for absorbance values of cases groups with and without silver nanoparticles application.

Parameter Group	Number of cases	Mean	Median	Variety	Standard deviation
Negative	10	0.818	0.845	0.008	0.088
Negative - Ag	12	0.809	0.821	0.006	0.074
Equivocal	26	1.015	0.995	0.014	0.118
Equivocal - Ag	5	0.940	0.920	0.002	0.046
Positive	44	2.036	2.162	0.310	0.557
Positive - Ag	63	1.823	1.801	0.380	0.617

diameter citrate capped silver nanoparticles application is given below (Table I). The basic statistical parameters (mean, median, variety and standard deviation) were calculated for absorbance values of cases groups that were interpreted as positive, equivocal, and negative with and without silver nanoparticles application (Table II). Application of silver nanoparticles increased absorbance values, correcting 19 equivocal cases as positive, 3 equivocal cases as negative and 1 negative case as equivocal.

Discussion

Our expectation was that application of silver nanoparticles[6] could enhance the signal relative to standard ELISA run. Accordingly, ELISA performed with silver nanoparticles should detect lower concentrations of IgG against nucleocapsid protein of SARS-CoV-2. To test this postulate, we compared standard ELISA test with the same test modified by addition of nanoparticle stock solution [mass concentration – 1.0 mg/mL. atomic (Ag) molarity – 9.27 mmol/L, molarity particle concentration – 1.5×10^{12} particles/mL, Ag mass percent (%) – 0.1, max optical density (cm^{-1}) – 120] at detection stage. In particular, nanoparticles were added 5 min advance of 3',3',5',5'-tetramethylbenzodine (TMB) substrate solution. We found that absorbance values were increased in ELISA run performed by application of nanoparticles. Although the physical and chemical interactions specific to nanoparticles interaction with antibodies or even their complexes were not considered in our study[7,8]. These aspects shall be investigated further.

Conclusions

As laboratory diagnostic methods must be sensitive enough to detect even trace number of biomarkers[9], our findings suggest that the application of nanoparticles can improve the sensitivity of ELISA method and have a positive impact on this method specific detection limit. Thus, it is logical and desirable to enhance the sensitivity of ELISA method applying nanoparticles; the approach is of low cost and positively impacts accuracy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval

The present study has been approved by the Bioethics International Committee of the Petre Shotadze Tbilisi Medical Academy. All procedures performed in the present study were in accordance with the Helsinki Declaration (as revised in 2013). The samples used in the present study were collected from the registered participants. The information about the study including the study design and objectives were presented to the potential participants together with the registration form.

Informed Consent

The participants were informed about the study design and objectives. All participants provided informed consent for inclusion and for anonymous data publication before they participated in the study.

Data Availability

The datasets created and analyzed during the current study are available from the corresponding author on reasonable request.

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Authors' Contribution

All authors contributed equally to this article. TM under supervision performed experimental activities; SI performed data analysis; EK contributed to data interpretation and manuscript preparation. All authors read and approved the final manuscript.

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