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Research Article

Abnormal Flotation of Separator Gel in Blood Test Tubes in the Hemodialysis Patients

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Abstract

Objectives: Serum-separating tubes (SST) are used in medical clinical chemistry tests requiring blood serum. Blood density is mainly dependent on the total protein concentration. Aim of this study to investigate, whether high protein content due to dehydration during hemodialysis can cause laboratory error with the abnormal flotation in blood tubes.

Methods: After examining post hemodialysis (HD) blood samples of three cases with ESRD we investigated the effect of adding bovine albumin to the test tubes to determine whether an increment in protein concentration result in abnormal flotation of separator gel in blood samples obtained from healthy volunteers.

Results: Serum total protein levels were markedly increased in blood samples of all 3 cases. In tests using blood samples from healthy volunteers supplemented with bovine albumin to increase the protein load, the test tubes having an albumin concentration greater than 16 g/dl displayed an abnormal flotation of separator gel similar to those we observed in post-HD samples from our cases.

Conclusion: Increased protein load, probably caused by Intradialytic hypotension due to dehydration, induces the observed abnormal flotation of separator gel in serum tubes in these cases. Our findings emphasize the importance of visual control of serum tubes to prevent abnormal flotation in blood samples.

Keywords: Abnormal flotation, hemodialysis, separtor gel, test tubes

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Specimen quality and time needed to obtain a proper specimen are important factors in the quality of laboratory results.[1] Serum separating tubes (SST) are used in medical biochemistry laboratories for performing tests in blood serum. These tubes containing a separator gel provide several advantages, such as; reduced sample manipulation and aerolization of hazardous substances and improvement in stability of serum for analysis.[2] The inert, thixotropic polymer gel (separator gel) content separates blood cells from serum by its gravity (1.04 g/cm^3) which lets the gel position in between the heavier (1.092 to 1.095 g/cm^3) cellular component and the lighter (1.026 to 1.031 g/cm^3) serum of the blood. After centrifugation, a barrier forms that prevents mixing of substances originated from celluler elements of the serum.[2, 3]

Although rarely, abnormal gel flotation due to an increase in plasma density may be observed. In cases such as hyperproteinemia[4] or following administration of a contrast dye analytical errors may result.[3] In our study, we present abnormal flotation of separator gel in blood samples of 3

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patients with end stage renal disease (ESRD), after completion of hemodialysis (HD) sessions. And researching the causes of this clinical condition in volunteers.

Methods

Blood samples from patients with ESRD before (pre-HD) and after (post-HD) completion of monthly HD sessions are drawn into BD SST II Advance Tubes (Becton Dickinson, NJ, USA) for analysis of routine biochemical parameters. After completion of coagulation, samples are centrifuged at 3000 rpm for 10 min and placed in Roche cobas 6000 c501 auto-analyzers for measurements. Here we describe 3 cases of abnormal flotation of separator gel in test tubes containing post-HD blood samples.

The first case of abnormal flotation of separator gel was discovered when we encountered a delay in delivery of the laboratory results due to bending and obstruction of the probe of auto-analyzer. Stucking of probe in separator gel on top of the test tube caused bending and obstruction of it.

Second and third cases occurred 1 week and 2 months after the first case, respectively, again only in post-HD blood samples (Fig. 1). In order to determine the reason(s) for the occurrence of abnormal gel flotation we reviewed the hospital records of the patients to determine the blood pressures of the patients. Furthermore, in samples with abnormal flotation of separator gel we measured the protein content and the biochemical parameters related to the density of the blood by gently removing the gel with a wooden spatula from the wall of the test tube and obtaining the serum by a Pasteur pipette.

In another set of experiments we investigated the effect of adding bovine albumin (Sigma A9647) to the BD SST II Advance test tubes. In these study, we aimed to determine whether an abnormal flotation of separator gel in blood samples causes falsely increased protein concentration in samples from healty persons (Fig. 2). Samples with gradually increasing protein concentrations were centrifuged at 3000 rpm for 10 min after a 30 min waiting period for coagulation.

The experimental procedure: The pool containing 40 g/dl total protein was prepared by adding bovine serum albumin to pool of serum. Informed consent was obtained from the persons (n=5) donating blood for the experimental study. First blood samples withdrawn from these volunteers were placed in tubes with separator gels and serum total protein levels were measured. Then after, second blood samples obtained from control subjects were placed in tubes and the total protein levels were adjusted approximately to 10 g/dL, 12 g/dL, 16 g/dL and 18 g/dL in serum of these samples by adding small amounts from the high protein serum pool. Tubes were centrifuged following a waiting duration of 30 min for coagulation. Following centrifuge, it was determined

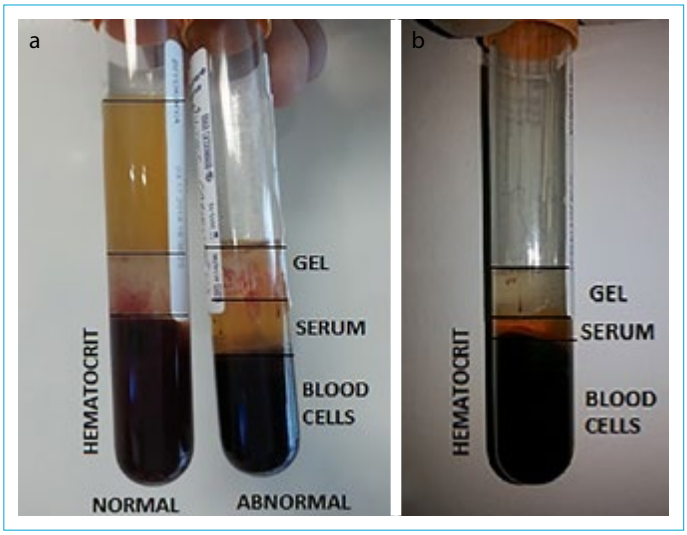

Figure 1. (a) Normal and abnormal placement of separator gel in blood sampling tubes. Following centrifugation together with increment in hematocrit ratio **(b)**.

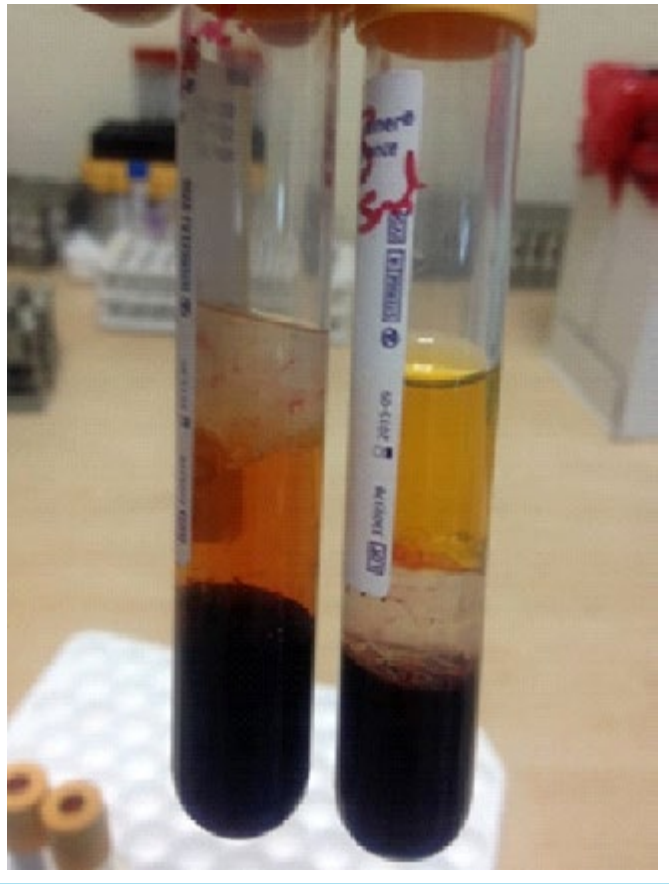

Figure 2. Normal (right) and abnormal (left) flotation of separator gel after centrifugation in blood samples from healthy volunteers with a protein load below (right) and above 16.16 gdl (left).

that the whole gel was on top of the serum in tubes with total protein levels of 16 g/dL and 18 g/dL. Mean total protein level was 16.16 g/dL in tubes that were calculated to contain approximately 16 g/dL protein.

The study was approved by the local ethics committee and written informed consent was received from all candidates.

Results

Characteristics of the three patients with ESRD were as follows:60 years old man; 68 years old woman and 48 years old man. (Note: Cases 2 and 3 also had type 2 diabetes mellitus).

A review of the hospital records revealed that all 3 cases with abnormal flotation of separator gel were hypotensive after completion of HD sessions. The abnormal flotations of separator gel were very similar to each other in all 3 cases and they occurred in post-HD blood samples.

The results of the biochemical measurements related to the density of blood in pre- and post-HD samples from the cases with abnormal flotation of separator gel was presented in Table 1.

Total protein levels of serum were markedly increased in post-HD blood samples of all 3 cases with abnormal flotation of separator gel (Table 1). Immunofixation electrophoresis demonstrated a marked increase in all protein fractions except the monoclonal one in post-HD, but not in the pre-HD blood sample of the second case (Fig. 3).

In experiments using blood samples from healthy volunteers supplemented with bovine albumin to increase the protein load, the test tubes having an albumin concentration greater than 16.16 g/dl displayed an abnormal flotation of separator gel similar to those we observed in post-HD samples from our cases with ESRD (Fig. 2).

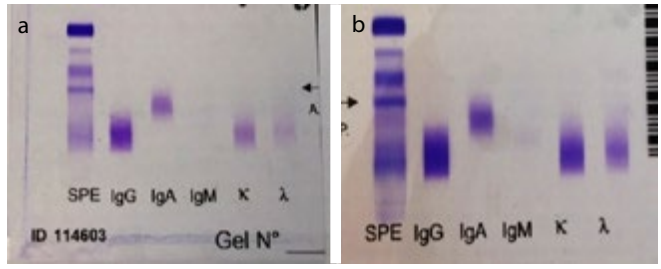

Figure 3. Immunofixation electrophoresis of pre-hemodialysis **(a)** and post-hemodialysis **(b)** blood samples from the second case with abnormal flotation of separator gel.

Discussion

Vacuum tubes containing separator gels are preferred in routine laboratory procedures because of the ease of use and the other advantages they provide. Silicone coatings of these tubes reduce adherence of red cells to wall of tube while micronized silica particles accelerate clotting. Following centrifugation, gel reaches a certain volume and forms a barrier between cells in blood and serum. It has been reported that many factors including tube material, temperature, centrifugation speed, storage and patient factors such as heparin therapy, elevated plasma protein, serum/plasma specific gravity and iodinated contrast media might be involved in abnormal flotation of separator gel on to the top of the test tube following centrifugation.[3, 5, 6]

HD has been used as part of the medical management of patients with ESRD for more than 50 years. Hydration of the patient deserves a specific consideration in management of patients on HD. Removal of intravascular volume by ultra-filtration comes dehydration and increased hematocrit levels due to shift of extravascular fluid compartment to intravascular space.[7–9] Intradialytic hypotension is estimated

Table 1. Blood pressures after completion of hemodialysis sessions in cases and results of the biochemical measurements performed in pre- (Pre-HD) and post-hemodialysis (Post-HD) blood samples from cases with abnormal flotation of separator gel

	Case 1		Case 2		Case 3	
Parameter	**Pre-HD**	**Post-HD**	**Pre-HD**	**Post-HD**	**Pre-HD**	**Post-HD**
Blood Pressure (mmHg)	120/70	90/55	110/60	80/50	110/70	90/50
Glucose (mg/dl)	95.2	ND	85	ND	129	ND
BUN (mg/dl)	109	1	102	2	63	1
Creatinine (mg/dl)	6.07	2.41	8.2	3.26	7.8	2.62
Potassium (mmol/l)	4.44	2.03	7.25	3.4	4.95	3.74
Total protein (g/dl)	6.68	17.1	7.7	16.49	6.5	16.28
Albumin (g/dl)	4.41	10.1	4.1	9.8	3.9	7.5
IgM (mg/dl)	55	144	ND	ND	ND	ND
IgG (mg/dl)	791	1809	1860	3959	ND	ND
CRP (mg/dl)	0.99	1.84	ND	ND	ND	ND

ND: Not determined.

to occur during 20%-30% of hemodialysis sessions.[10]

There is no generally accepted definition of intradialytic hypotension. Kidney Disease Outcomes Quality Initiative (K/DOQI) and European Best Practice Guidelines define intradialytic hypotension as the presence of a decrease in systolic blood pressure ≥20 mmHg or a decrease in mean arterial pressure by 10 mmHg, which is associated with clinical events and need for nursing interventions.[11]

Blood volume is dependent on two main factors: plasma refilling capacity and UF rate. Plasma refill is important for cardiovascular stability during haemodialysis treatment. During HD fluid is removed from the vascular space during ultrafiltration (UF). There is redistribution of fluid from the exstravascular pool the intravascular compartment and this is known as plasma refilling.[12] For instance, both a high interdialytic weight gain and a short treatment time usually result in more aggressive UF, which in turn will be followed by an imbalance of the UF/refill ratio even in normovolaemic patients.[13] Hypovolemia can result when the UF rate exceeds the plasma refilling rate. Thus, not only the large and rapid ultrafiltration but the factors diminishing the plasma refilling rate are what exacerbate the hypovolemia.[14]

In accordance, a careful analysis of hospital records revealed that all our cases with abnormal flotation of separator gel were hypotensive during the collection of post-HD blood samples. We observed the flotation of separator gel only in post HD patients with normal blood pressure. The pre-HD period further supported a contributory role of intradialytic hypotension in the observed error in pre-analytical phase. Besides that, visual examination of samples revealed increased hematocrit ratios due to hemoconcentration.

It has been reported that the increased protein content was the cause of abnormal flotation of separator gel in two patients with multiple myeloma.[15] In another study by Faught et al.[16] it has been suggested that abnormal flotation of separator gel might be seen in clinical samples with protein contents of more than 14.06 g/dl and 16.2 g/dl in Greiner and BD test tubes, respectively. In accordance, we found marked increased protein contents, exceeding 16.28 g/dl in post-HD blood samples from all our cases (Table 1). Taken together, these findings suggested that the extent of intradialytic hypotension-induced increment in protein load of blood rather than the specific disease itself play a more important role in abnormal flotation of separator gel in patients with ESRD. Induction of the same abnormality, experimentally, by increasing the protein content of blood samples from healthy volunteers to a level above 16.16 g/dl by adding bovine albumin to test tubes further supported this view.

In pre-analytical automation systems of clinical labs with a heavy burden of workload and sample volume, possible errors derived from test tubes are prevented by the warnings of analytical devices. However, in laboratories without this system it depends on the vigilance of lab technicians. In our cases, following the first experience, we informed lab staff about the event and the importance of visual control of the tubes before placement in auto-analyzer. This corrective and preventive action resulted in recognition of the test tubes with similar abnormal flotation of separator gel before placement in auto-analyzer in the second and third cases, which occurred 1 week and 2 months after the first case, respectively.

Conclusion

Present findings emphasize;

1. the limitations of test tubes with separator gels in cases with increased protein load of blood samples;
2. the importance of visual control of test samples before placement into autoanalyzers in order to avoid some preventable errors in pre-analytical phase.

Clinical laboratory teams should be aware of possibility of abnormal flotation of separator gel due to increased protein load in samples especially at patients with volume loss. In order to ensure that, the center delivering these samples must be contacted to get necessary clinical information about co morbidities. Control algorithms to prevent occurrence of intradialytic hypotension in patients with ESRD might help in prevention of abnormal flotation of separator gel.

Taken together these measures might help clinical labs, with a heavy burden of workload, in preventing time, workload and economical losses due to limitations of serum tubes with separator gels.

Disclosures

Ethics Committee Approval: Antalya Medical Park Hospital Complex, no: 2018/04.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept - M.D.; Design - M.D., S.O.; Supervision - S.O.; Material - M.D.; Data collection - M.S.; Analysis - M.D.; Literature search - M.S.; Writing - M.D.; Ciritical review - S.O.

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