EJCRIM
European Journal
of Case Reports
in Internal Medicine

INADEQUATE ABSORPTION OF INTRAMUSCULAR ANTI-D IMMUNOGLOBULIN IN A PREGNANT WOMAN WITH A HIGH BODY MASS INDEX

Ivica Marić, Klara Železnik

Slovenian Institute for Transfusion Medicine, Ljubljana, Slovenia

Corresponding author's e-mail: ivica.maric@ztm.si

Received: 26/09/2025
Accepted: 07/10/2025
Published: 24/11/2025

Conflicts of Interests: The Authors declare that there are no competing interests.
Patient Consent: Written informed consent was obtained from the patient for the publication of this case report and any accompanying images.
Acknowledgments: The authors would like to thank the clinical and laboratory staff of Slovenian Institute for Transfusion Medicine for their support in patient management and data collection.
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How to cite this article: Marić I, Železnik K. Inadequate absorption of intramuscular anti-D immunoglobulin in a pregnant woman with a high body mass index. *EJCRIM* 2025;**12**:doi:10.12890/2025_005868

ABSTRACT

Introduction: The D antigen is highly immunogenic, and alloanti-D antibodies in maternal blood may cause haemolytic disease of the foetus and newborn. Prophylactic administration of anti-D immunoglobulin (Ig) to D-negative pregnant women significantly reduces the risk of alloimmunisation. However, absorption after intramuscular (IM) injection may be impaired in obese women.

Case description: We report the case of a 27-year-old D-negative pregnant woman body mass index (BMI) 35 kg/m^2 who received three IM injections of anti-D Ig during pregnancy and after caesarean section. Despite confirmed administration, her indirect antiglobulin tests (IATs) remained negative or only borderline positive. Investigations excluded weak/partial D variants, foetomaternal haemorrhage, chimerism, and other causes. Due to her high BMI, we hypothesised that the injections were administered into subcutaneous fat, resulting in minimal absorption. After intravenous administration of anti-D Ig, the IAT became strongly positive, confirming correct drug delivery.

Conclusion: This case highlights the importance of considering maternal BMI in the administration of anti-D Ig. In obese women, inadequate IM administration may compromise prophylaxis. Intravenous administration or the use of alternative injection sites should be considered.

KEYWORDS

D antigen, alloimmunisation, anti-D immunoglobulin, intramuscular injection, obesity

LEARNING POINTS

- Subcutaneous tissue thickness plays a crucial role in the absorption of intramuscular anti-D immunoglobulin.
- Women with high body mass index are at risk of inadequate intramuscular administration, which may result in subtherapeutic serum anti-D levels.
- The intravenous route or careful selection of injection site (e.g., deltoid) should be considered in obese patients.

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INTRODUCTION

The D (RH1 or RhD) antigen is one of the most clinically significant antigens of the Rh blood group system and is known for its strong immunogenicity. When a D-negative mother is exposed to D-positive red blood cells (RBCs), either during pregnancy or delivery, there is a considerable risk of maternal alloimmunisation. The maternal immune system can mount a response, producing alloanti-D antibodies that readily cross the placenta. These antibodies target foetal D-positive RBCs, leading to haemolysis. The clinical spectrum of haemolytic disease of the foetus and newborn (HDFN) ranges from mild neonatal jaundice to severe anaemia, hydrops foetalis and, in the most severe cases, intrauterine death[1,2].

Before the introduction of routine immunoprophylaxis, D alloimmunisation was one of the leading causes of perinatal morbidity and mortality worldwide. The development and widespread use of anti-D immunoglobulin (Ig) prophylaxis since the late 1960s has been a landmark achievement in obstetric and transfusion medicine, reducing alloimmunisation rates dramatically, to below 0.1% in most developed countries. The exact mechanism of anti-D Ig is not fully understood but is thought to involve binding to foetal D-positive RBC epitopes in maternal circulation and promoting their clearance by macrophages before immune recognition. Despite this success, occasional failures still occur. These may result from inadequate or delayed dosing, missed sensitising events, rare immunological mechanisms, or impaired absorption of intramuscularly administered preparations. Increasing attention has recently been paid to maternal factors such as obesity and body mass index (BMI), which may affect the pharmacokinetics of anti-D Ig and reduce its clinical efficacy. This highlights the ongoing need for vigilance and optimisation of administration strategies, even in the era of highly effective prophylaxis[1,2].

In Slovenia, legislation requires that all pregnant women undergo blood grouping and an indirect antiglobulin test (IAT) using column agglutination technology by the 12th week of gestation. At 25–26 weeks, foetal RHD genotyping is performed from maternal plasma. If the foetus is predicted to be D-positive, IAT is repeated before an intramuscular (IM) injection of anti-D Ig is administered at 28 weeks. Postpartum, an additional dose is given within 72 hours if the neonate is D-positive. Additional doses are indicated after sensitising events[3].

Anti-D Ig is derived from pooled plasma of alloimmunised donors and is available in Slovenia in two formulations. According to manufacturer guidelines, route of administration (IM vs intravenous, IV) and maternal BMI should be considered[4,5].

CASE DESCRIPTION

A 27-year-old D-negative woman, gravida 1, presented at 22 weeks of gestation with minor vaginal bleeding. IAT was negative, and she received 1500 IU anti-D Ig IM.

At 26 weeks, she presented again with bleeding. Emergency caesarean section was performed due to premature rupture of membranes and cord prolapse. The neonate was stillborn. IAT was negative, and a second dose of 1500 IU anti-D Ig was administered IM.

Four days postpartum, she developed anaemia and received two units of packed RBCs. IAT was negative, and she received a third IM dose of 1500 IU anti-D Ig. The following day, IAT was borderline positive. Repeated IATs and papain-enhanced testing showed only weak positivity.

Further investigations were undertaken to clarify why the patient's IAT remained only borderline positive despite confirmed administration of anti-D Ig. RHD genotyping confirmed the patient was truly D-negative, excluding weak or partial D antigen expression that might have absorbed the administered Ig. The direct antiglobulin test was negative. A large foetomaternal haemorrhage was excluded by Kleihauer–Betke testing and by flow cytometry (FC) using anti-HbF monoclonal antibodies. FC with anti-D monoclonal antibodies also excluded red cell chimerism (the presence of a small population of D-positive RBCs among predominantly D-negative RBCs), which would not have been detected by routine serology, as well as foetomaternal haemorrhage (FMH) from a D-positive foetus (the foetal blood group was not known). In summary, potential causes that might have neutralised the administered anti-D Ig—maternal D variant, red cell chimerism, or significant FMH—were excluded.

Anthropometric data revealed BMI 35 kg/m^2 (height 167 cm, weight 98 kg). It was hypothesised that injections were administered into gluteal fat, resulting in poor absorption. A further 1500 IU anti-D Ig was administered IV Four hours later, IAT was strongly positive, and this persisted the following day, confirming effective administration.

DISCUSSION

Alloimmunisation to the D antigen remains a concern despite prophylaxis with anti-D Ig, owing to the pronounced maternal–foetal antigen disparity and the strong immunogenicity of the D antigen. Appropriate administration of anti-D Ig reduces the risk of alloimmunisation to less than 0.1%[1]. While numerous studies have compared single postpartum dosing with combined antepartum and postpartum regimens, only a limited number have investigated the IM versus IV routes of administration. Even fewer studies have addressed the influence of maternal BMI, body weight, or body surface area (BSA) on the absorption and pharmacokinetics of anti-D Ig.

Evidence suggests a correlation between maternal obesity and reduced serum concentrations following IM administration. The most likely explanation is the increased thickness of subcutaneous tissue in overweight women, which impedes effective delivery of the drug into muscle. Chan et al.[6] demonstrated the impact of BMI and subcutaneous tissue depth on IM. drug delivery: in only 8% of women was the injection successfully administered into the gluteal muscle, with the remainder deposited in adipose tissue. Greater subcutaneous thickness with higher BMI was

associated with reduced efficiency of IM delivery[6]. Similarly, Zaybak et al. confirmed these findings, concluding that a needle length of at least 3.8 cm is necessary for effective IM administration in women with a BMI above 24.9 kg/m^2[7].

The site of injection also plays a critical role. The deltoid muscle is considered both safe and appropriate for IM administration of anti-D Ig and may be preferable to the gluteal region in overweight women. When the gluteal site is used, care must be taken to ensure that the injection reaches muscle, as absorption may otherwise be delayed if the preparation remains within subcutaneous fat. It remains unclear, however, whether this translates into an increased sensitisation rate in overweight women[8].

Pharmacokinetic studies further support these concerns. Bichler et al. showed that women weighing more than 80 kg required longer to reach peak serum anti-D concentrations after IM administration, and their levels were lower compared with women weighing less than 80 kg[9]. Mackenzie et al. demonstrated a significant association between higher maximum serum anti-D levels and lower maternal BSA, although this did not influence the duration of detectable Ig following IM injection[10]. Woelfer et al. concluded that higher BMI was associated with a reduced rise in serum anti-D following IM injection, with the effect becoming significant when BMI exceeded 27 kg/m^2. Importantly, the relationship was progressive rather than linear: mean peak levels 72 hours post-injection were 28–60% lower in women with BMI 28–40 kg/m^2 compared to those with BMI ≤27 kg/m^2. Lower concentrations may also reflect the larger circulating blood volume in obese women[11].

Recognition of the potential for reduced efficacy of IM anti-D administration in women with high BMI has therefore emerged from several pharmacokinetic studies[9–11]. These consistently show lower peak serum levels in overweight women, though the clinical significance remains uncertain. To date, published evidence has not established a direct association between reduced serum concentrations and an increased risk of alloimmunisation. As such, no firm recommendations can be made regarding the routine use of higher doses or IV administration in women with high BMI. Nonetheless, caution is warranted when administering anti-D Ig intramuscularly in this population, with careful selection of the injection site and needle length to ensure the preparation reaches muscle tissue[8]. Additionally, Ngan et al., in their meta-analysis, examined the association between BMI and anti-D concentrations in D-negative pregnant women. Three studies were identified that reported a negative correlation between increasing BMI and blood concentrations of anti-D prophylaxis, which lends further support to our assertion that impaired absorption in our case report is related to the patient's high BMI[12].

Finally, it is important to note that the precise mechanism of action of anti-D Ig is not fully understood. Its effect may relate less to maternal serum levels and more to the interaction between administered Ig and the number of circulating foetal red blood cells. Consequently, its mode of action may not be directly analogous to therapeutic drug levels[13].

CONCLUSIONS

This case illustrates the impact of obesity on the absorption of IM anti-D Ig. In women with high BMI, IM administration into gluteal fat may lead to inadequate serum levels. Consideration should be given to alternative injection sites or the IV route.

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