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Project: Vitamin C status of patients with severe infection and obesity

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Introduction:
Vitamin C is an essential vitamin with antioxidant and anti-inflammatory properties. The human body has lost the ability to synthesise its own vitamin C, we therefore rely fully on our diet to obtain adequate vitamin C levels. It also means that our body's vitamin C stores can take a severe hit when we become unwell. Due to oxidative stress, conditions such as a severe infection like pneumonia and obesity can compromise vitamin C levels as a result of the disease process.

This study was designed to measure the vitamin C levels of blood samples taken from patients with either of these two conditions and to correlate their vitamin C levels with disease severity and patient outcomes. Because vitamin C is sensitive to oxidation and this study is being done retrospectively, it is important to test the stability of vitamin C in samples that have been handled and stored under different conditions.

Aim:
The key aims of this research include:
1. To investigate the stability of vitamin C in blood plasma stored under different conditions.
2. To measure the vitamin C status of patients with pneumonia (VIDCAPS study: admission and 6 weeks).
3. To measure vitamin C status of patients undergoing bariatric surgery (at surgery, and follow-up at 1, 3, 6, 12 months).
4. To correlate patient vitamin C levels with markers of disease severity and patient outcomes.

Method:
In vitro experiments investigated the stability of vitamin C in Phosphate Buffered Saline (PBS) stored for up to 24 hours at room temperature, at 4°C and on ice. Loss of vitamin C in these samples was measured using UV Spectrometry. Analysis of vitamin C in a biological samples (blood plasma) was determined using High Performance Liquid Chromatography (HPLC) with electrochemical detection (ECD) which separates the biomolecules within a sample with interactions with a column and vitamin C is then electrochemically detected. Recovery of oxidised vitamin C by reaction with reducing agent TCEP was also optimised using this method.

Results:
Our vitamin C stability experiments show that in PBS vitamin C is stable at 4°C for up to 6 hours but experiences a 20% decrease over 24 hours and vitamin C is unstable at room temperature with complete loss by 24 hours. Our studies performed in blood plasma show that in room temperature vitamin C is very unstable and is rapidly and completely lost within 4 hours. Vitamin C in plasma that has been kept at 4°C also decreases within a few hours, but this may be recovered with a reducing agent, TCEP. However, after 24 hours at 4°C little vitamin C can be recovered by TCEP.

Conclusion:
We found that, due to sensitivity to light, temperature, and oxygen, vitamin C-containing blood samples need to be processed at 4°C and then frozen at -80°C within a couple of hours. These results indicated that the vitamin C in the pneumonia cohort is likely to have been lost due to incorrect handling of the samples. A couple of preliminary pneumonia samples that have been run through HPLC-ECD confirm this. In contrast, the bariatric samples have been handled and stored appropriately for vitamin C analysis, so these will be analysed for their vitamin C content.

These findings highlight the difficulty of carrying out retrospective studies of vitamin C and also show the importance and clinical relevance of proper handling and processing of samples for vitamin C analysis.

From this research we will be able to publish a paper that will inform researchers of the best processing methods for vitamin C, which will improve future research.