International Journal of Medical Science and Dental Research

# Assessment of Some Haematological Parameters Using Varying Concentrations of EDTA

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**ABSTRACT:** Studies on the effects of varying concentrations of EDTA was investigated on packed cell volume (PCV), total white blood cell count (TWBC) and platelet count. The research was carried out on blood samples collected into different concentrations of EDTA to evaluate a change on these parameters. The mean values for each concentration were calculated, and at optimal concentration of 10%, PCV was 0.44±1.4, TWBC was 5.6±1.6 x $10^9$/L and platelet was 224±34 x $10^9$/L. It was observed that at 6% and 8% concentration, there were no significant effects of EDTA on these parameters, whereas at 12% and 14% concentration, significant changes were observed on these parameters at probability level $p<0.05$. This study therefore advocates strict adherence to 10% concentration of EDTA.

***Keywords:*** *PCV, TWBC, Platelet, varying concentrations of EDTA*

## I. INTRODUCTION

Ethylene diamine tetra acetic acid, widely abbreviated as EDTA, is a chemical compound synthesized from 1, 2-diaminoethane, formaldehyde, sodium cyanide and water. (Vandermee *et al.*, 2002).

It is widely used as an anticoagulant. Anticoagulants are substances that prevent blood from clotting (Baker *et al.*, 2001). They preserve blood when used in the right proportion.

They could be in liquid form or in solid form. Their action on blood is based on their chemical composition. I In the specific field of in vitro diagnostics, anticoagulants are commonly added to collection tubes either to maintain blood in the fluid state for hematological testing or to obtain suitable plasma for coagulation and clinical chemistry analyses. Unfortunately, no universal anticoagulant could be used for evaluation of several laboratory parameters in a sample from a single test tube available so far.

Ethylene diamine tetra acetic acid (EDTA) is a polyprotic acid containing four carboxylic groups and two-amine group with; lone pair electrons that chelate calcium and several other metal ions. Calcium is necessary for a wide range of enzyme reactions of the coagulation cascade and its removal irreversibly prevents blood clotting within the collection tube. EDTA has been recommended as the anticoagulant of choice for hematological testing because it allows the best preservation of cellular components and morphology of blood cells. (Giuseppe *et al.*, 2007). In contemporary hematological analysis, however, there are numerous cases of erroneous results

from in appropriate usage of EDTA concentration due to collection of less or more volume of blood into EDTA bottle with a specified concentration and specified volume of blood or high concentrations of EDTA, more than is required for a particular amount of blood. And like any other chemical substance, EDTA is capable of inducing gross changes in blood components, which affects hematological parameters.

## II. OBJECTIVES

This research project aims to

1. Estimate packed cell volume (PCV), total white blood cell counts (TWBC), and platelet count on varying concentrations of EDTA.

2. Establish the optimal concentration of EDTA for hematological analysis.

The intents of this work therefore are to study the effects of varying concentrations of EDTA on hematological parameters and cell morphology.

## III. MATERIAL AND METHODS

**STUDY SITE AND POPULATION**

The site used for this study was Ebonyi State University and its environs, and a total of 11 samples were collected. The tests were carried out at Ebonyi State University Teaching Hospital.

**REAGENTS USED**

- Ethylene diamine tetra acetic acid (EDTA)
- Ammonium oxalate
- Turk's solution

**METHODS**

**SAMPLING AND SAMPLE SIZE.**

The subjects used the research project were selected randomly and a total of 11 samples were used.

**ETHICAL CONSIDERATION**

Permission was sought and got from the subjects and also from University Teaching Hospital.

**PREPARATION OF EDTA ANTICOAGULANT**

Ethylene diamine tetra acetic acid used was produced by BDH Chemicals Ltd, Poole, England. 100g

The concentrations prepared were 6%, 8%, 10%, 12% and 14% Preparations are as follows;

- **6% CONCENTRATION**
  - 6 grams of EDTA powder was measured, using a weighing balance.
  - 100ml of distilled water was measured, using a rjieasuring cylinder. The 6 grams of EDTA was added into the 100ml of distilled water and dissolved.
  - A drop was added to each plain tube, labeled 6% concentration.

- **8% CONCENTRATION**
  - 8% grams of EDTA powder was measured with a weighing balance
  - 100ml of distilled water was measured, using a measuring cylinder
  - The 8g of EDTA was added to the distilled water and dissolved.
  - A drop was added to each plain tube, labeled 8% concentration.

- **10% CONCENTRATION**
  - 10g of EDTA was measured and dissolved in 100ml of distilled water
  - A drop was added to plain tube, labeled 10% concentration

- **12% CONCENTRATION**
  - 12g of EDTA was dissolved in 100ml distilled water
  - A drop was added to plain tube labeled 12% concentration

- 14% CONCENTRATION
  - 14g of EDTA was dissolved in 10ml distilled water and a drop added to plain tube labeled 14% concentrations.

**COLLECTION OF BLOOD SAMPLES**

Blood was drawn from a vein (venepuncture), the medial cubical vein. A soft tubing tourniquet was fastened to the upper arm of the subject to enable the veins to be seen and felt. The tourniquet was not applied to tightly and the subject was asked to make a tight fist to make the veins more prominent. The index finger was used to feel for a suitable vein, and a sufficiently large vein was selected. The puncture site was cleansed with 70% ethanol and allowed to dry. With the thumb of the left hand holding down the skin below the puncture site, the venepuncture was made with the bevel of the needle directed upwards in the line of the vein. The plunger of the syringe was steadily withdrawn at the speed it was taking the vein to fill. When sufficient blood had been collected, the tourniquet was release and the subject was instructed to open his fist. The needle was removed and immediately the puncture site was pressed with a dry cotton wool. The tourniquet was completely removed and subject was instructed to continue pressing the puncture site until bleeding stopped. The needle was carefully remove from the syringe and discarded safely.

10mls of blood was collected from each subject, and 2rnls were added in to the tubes, labeled 6%, 8%, 10%, 12% and 14% each tube containing a drop of anticoagulant of the labeled concentrations.

**PACKED CELL VOLUME (PCV)**

(Method adopted was that of Cheesbrough, 2004)

**PRINCIPLE**

The packed cell volume (PCV) is that proportion of whole blood occupied by red cells expressed as a ratio (Litre/ Litre) Anticoagulant blood in a glass capillary of specified length bore size and wall thickness is centrifuged in a microhematocrit centrifuge at RCF 12000-15000xg for 3-5minutes to obtain constant packing of the red cells. A small amount of plasma remains trapped between the packed red cells. The PCV value is read from the scale of a microhematocrit reader of calculated by dividing the height of the red cell column by the total column of blood.

**PROCEDURE**

1. A plain capillary tube was filled with well mixed EDTA anticoagulant blood to the three quarter mark.
2. The unfilled end was sealed using a sealant material.
3. The filled capillary tube was carefully located in one of the numbered slots of the microhaematocrit centrifuge with the sealed end touching the rim of the gasket.
4. The tube was centrifuged for 5minutes at 12000 RCF.
5. The PCV was read using the microhaematocrit reader.

**WHITE CELL COUNT**

(Method adopted was that of Cheesbrough, 2004)

**PRINCIPLE**

Whole blood is diluted I in 20 in an acid reagent that haemolyses the red cells (not the nucleus of nucleated red cells), leaving the white cells to be counted white cells are counted microscopically using an improved Neubauer ruled counting chamber (haemocytometer) and the number of white blood cells per litre of blood is calculated.

**PROCEDURE**

1. 0.38ml of Turks solution was measured and dispensed into a small tube.
2. 0.02ml of well mixed EDTA anticoagulant blood was added and mixed.
3. The counting chamber was assembled. The central grid areas were ensured to be clean and dry. The cover slip was pressed down to each side of the grid areas until Newton rings were seen.
4. The diluted blood sample was remixed and a capillary tube was used to fill the grid area of the chamber.
5. The chamber was left undisturbed for 2 minutes to allow white cells to settle

39

6. The underside of the chamber was dried and the chamber was placed on the microscope and the white cells were counted.

**PLATELET COUNT**

(Method adopted was that of (Cheesbrough, 2004)

**PRINCIPLE**

Blood is diluted 1 in 20 in a filtered solution of ammonium oxalate, which lyses the red cells. Platelets are counted microscopically using an improved Neubauer ruled I counting chamber and the number of platelets per litre of blood calculated.

**PROCEDURE**

1. 0.38ml of filtered ammonium oxalate was measured and dispended into a small tube.
2. 0.02ml of well mixed EDTA anticoagulant blood as added and missed.
3. The counting chamber was assembled. The central grid areas were ensured to be clean and dry. The cover slip was pressed down to each side of the grid areas until Newton rings were seen.
4. The diluted blood sample was remixed and a capillary tube was used to fill the grid area of the chamber.
5. The chamber was left undisturbed for 20 minutes to allow platelets to settle.
6. The underside of the chamber was dried and the chamber was placed on the microscope and the platelets were counted.

**STATISTICAL ANALYSIS**

Student's T-test analysis was used to analyze the data obtained

## IV. RESULTS

Packed cell volume (PCV), Total white blood cell count (TWBC), and platelets were estimated at varying concentrations of EDTA.

The mean concentrations of each of the different concentrations were calculated from the result of the blood samples. The mean PCV from 6% to 14% concentration were; 0.36 ± 1.4, 0.43 ± lX.0.44 ± 1.4, 0.40 ± 1.4 and 0.37 ± 1.4 respectively all in L/L.

Also the mean TWBC from 6% to 14% were, 5.2 ± 1.6, 5.4 ± 1.6, 5.6 ± 1.6, 6.1 ±1.6 and 7.1 ± 1.6 respectively, each of the mean values multiplied by $10^{0}$.The mean platelet from 6% to 10% ranged from 219 ± 34 x $10^{9}$ to 224 ± 34 x $10^{9}$, while at 12% and 14%, they were 234 ± 34 x $10^{9}$ and 234 ± 34 x $10^{9}$ respectively.

The results of the above interpretation are summarized on Table 4.2, which shows the variations in concentrations of EDTA in mean PCV, TWBC and platelet of blood samples.

**Table** 2 shows the comparison of PCV, TWBC and platelet values at different concentrations of EDTA with 10% as a control, using the student T test and probability (p) level of 0.05.
From the table, it is observed that in EDTA concentration from 6% to 14%, there were significant differences in PCV, TWBC and platelet values as the tabulated value were less than the calculating values, (i.e. Teal > T tab) and at these concentrations, the p value is greater than o.05 (i.e. p>0.05) which indicates significant increased in PCV, TWBC and platelet at 5% level of significance.

**Table 1:** The effects of varying concentrations of EDTA ON PCV, TWBC and Platelet count.

| Concentration | PCV L/L | TWBCX$10^7$L | PLATELET |
|---|---|---|---|
| X$10^7$L | - | | |
| % | X±S.D | X±S.D | X±S.D |
| 6 | 0.36 ± 1.4 | 5.2 ± 1.6 | 219 ± 34 |
| 8 | 0.43 ± 1.4 | 5.2 ± 1.6 | 222 ± 34 |
| 10 | 0.44 ± 1.4 | 5.6 ± 1.6 | 224 ± 34 |
| 12 | 0.40 ± 1.4 | 6.1 ± 1.6 | 234 ± 34 |
| 14 | 0.37 ± 1.4 | 7.1 ± 1.6 | 247 ± 34 |

**Table 2:** Comparison of PCV, TWBC and Platelets at varying EDTA concentration using 10% as control

| Concentration | PCV | L/L | | TWBN X$10^9$/L | | | Platelet X$10^9$/L | | |
|---|---|---|---|---|---|---|---|---|---|
| % | Cal | Tab | P | Cal | Tab | P | Cal | Tab | P |
| | Ratio | Ratio | | Ratio | Ratio | | Ratio | Ratio | |
| 10% vs. 6% | 16.25 | 2.2.3 | 0.05 | 5.95 | 2.2.3 | 0.05 | 17.41 | 2.2.3 | 0.05 |
| 10 vs. 8% | 16.75 | 2.2.3 | 0.05 | 7.42 | 2.2.3 | 0.05 | 17.41 | 2.2.3 | 0.05 |
| 10% vs. 12% | 10.64 | 2.2.3 | 0.05 | 10.32 | 2.2.3 | 0.05 | 2.59 | 2.2.3 | 0.05 |
| 10% vs. 14% | 7.32 | 2.2.3 | 0.05 | 4,55 | 2.2.3 | 0.05 | 4.19 | 2.2.3 | 0.05 |

## V. DISCUSSIONS AND CONCLUSION

Three hematological parameters were estimated to assess at the aim of this research project namely, PCV, TWBC, and Platelet, at varying concentration of EDTA; 6%, 8% 10%,, 12% and 14% respectively.
The mean concentrations of each of the different concentrations were calculated from the result of the blood samples. The mean PCV from 6% to 14% concentration were; 0.36 ± 1.4, 0.43 ± 1.4, 0.44 ± 1.4, 0.40 ± 1.4 and 0.37 ± 1.4 respectively all in L/L.
Also the mean TWBC from 6% to 14% were, 5.2 ± 1.6, 5.4 ± 1.6, 5.6 ± 1.6, 6.1 ± 1.6 and 7.1 ± 1.6 respectively, each of the mean values multiplied by $10^9$
The mean platelet from 6% to 10% ranged from 219 ± 34 xl$0^9$ to 224 ± 34 x $10^9$, while at 12% and 14%, they were 234 ± 34 x $10^9$ and 247 ± 34x $10^9$ respectively.
The results obtained in this project research corresponded with the results of other works.
It was observed that EDTA concentration above 10% decreased the packed cell volume (PCV) and at these concentrations, white cell counts and platelet counts were higher.
The fall in PCV was due to the hypertoncity of plasma, causing water a to move from cells into the plasma, resulting in a reduction in cell size and finally shrinkage due to an excessive concentration (Faggio *et al*.,2014; Okoroiwu *et al.*, 2014). There was also swelling and disintegration of platelets and leukocytes at concentrations higher than 10%, due to excess concentration of EDTA in blood, resulting in high white all count and platelet count (Lippincolt, 1992).
Concentrations lower than 10% had no significant effects on these parameters.

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