Chapter 11
Hematological Complications and Rouleaux Formation of Blood Components (Leukocytes and Platelet Cells) and Parameters

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Abstract This chapter has the detailed and depth knowledge about the hematological complications and Rouleaux formation of blood components (leukocytes and platelet cells) and parameters. The purpose of this chapter is to determine the changes in three parameters, i.e., cells count, shape of cells, and size of cells, prior and after addition of three analytes, i.e., sugar, sodium chloride, and pure water, for ten varying concentrations, i.e., from 0 to 450 mM, admixed in 2 ml blood for sugar [$C_6H_{12}O_6$], 3 ml blood for sodium chloride [NaCl] and 4 ml blood for pure water. We have also discussed the effects of sugar, salt, and distilled water in comparison with the preexisting literature. This chapter also contains information's about sample preparation; methodology of bright and dark field microscopy under transmission mode used for each blood cells and parameters, 2-D images of each phantom of each analyte for its normal sample and admixed sample, tables and graphs to express the variation in parameters relative to each phantom, results of each phantom and detailed discussions about changes in blood parameters and components mentioned above for each analyte.

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Abbreviations

HGB	Hemoglobin
RDW	Red blood cells distribution width
HCT	Hematocrit
MCV	Mean corpuscular volume
MPV	Mean platelet volume
RBCs	Red blood cells
PDW	Platelet distribution width
PCT	Platelet crit
PRP	Platelet rich plasma
WBCs	White blood cells

11.1 Introduction

Blood is a fluid in our body which is not only responsible for transportation of nutrients (proteins, fats, minerals, carbohydrates, etc.) but also waste materials (carbon dioxide) out of body [1]. Blood is composed of plasma and cells. Blood cells are red blood cells (erythrocyte), white blood cells (leukocytes), and platelet cells (thrombocytes) [2]. Each male contains 4.7–6.1 million and female contains 4.2–5.4 million erythrocytes in one microlitter, 4×10^3 to 11×10^3 leukocytes and 2×10^5 to 5×10^5 thrombocytes [3, 4]. Normally, two to three drops have 1 billion RBCs with a ratio 600:1:40 of RBCs, WBCs, and platelets [3]. This chapter has the detailed and depth knowledge about the effects of sugar, salt, and pure water on shape, size; blood parameters and cells count of both leukocytes and thrombocytes. Many laboratory techniques and methods can be used for this type of biological and hematological study such as optical diffuse reflectance (ODR) [5], mid infrared spectroscopy (MIS) [6], light polarimetry [7], Raman spectroscopy [7], photo acoustic spectroscopy (PAS) [8], computed tomography (CT), magnetic resonance imaging (MRI) [9], optical coherence tomography (OCT) [10], and light microscopy [11]. Every method has its own advantages but light microscopy has many advantages over other techniques like easy to take it from one place to another, live observation of cells or bodies with no harmful radiations, cheap without little exception, has high magnification power. It gives information's about biological activities and parameters such as cell division, cell movement, shape and size of cells, pigments, etc. There exist many microscopic techniques like infrared microscopy (IRM), scanning probe microscopy (SPM), ultraviolet microscopy (UVM),) digital holographic microscopy (DHM), digital pathology (virtual microscopy), laser microscopy (LM), optical microscopy (OM), electron microscopy (EM) and optical microscopy (OM) and amateur microscopy (AM) [11], but we used light microscopic technique (bright and dark field microscopy) under transmission mode. In our work, we use light microscopy to diagnose blood cells

and parameters, i.e., white light microscopy for WBCs and PRP under dark field microscopy for platelet cells. This type of microscopy uses beam of light that makes fluorescence in sample or slide, transmitted through it, and one can observe many times magnified view of cells through eyepiece.

11.2 Materials and Methods

In this research work, we have used two types of samples in order to diagnose the blood parameters and blood cells (leukocytes and thrombocytes only).

11.2.1 Sample Type I for Leukocytes

To diagnose the WBCs parameters, we used microscope under transmission mode for aforementioned ten concentrations of sugar, sodium chloride, and pure water from 0 to 450 mM range with difference of every 50 mM. We poured 2 ml blood for sugar, 3 ml for sodium chloride, and 4 ml for pure water in ten different heparin tubes. Heparin is used as anticoagulant. Then, we mixed each concentration of every analyte with a difference of every 50 mM in each heparin tube. Heparin works as anticoagulant agent. 0 mM means no extra analyte is admixed in first sample. Blood smear of each analyte sample was prepared by using ethanol as fixing agent and field strain (A, B) for staining. Slide for each sample was then examined after putting one drop of emersion oil under microscope model (Olympus CX41) at 100×. The images of each sample set were taken with digital camera model (Canon EOS 600D, Japan). Whole experiment was conducted at room temperature.

11.2.2 Sample Type II for Platelet Cells

Blood with blood group (**AB+)** of a healthy person was taken into ten EDTA tubes for each analyte. EDTA is an anticoagulant agent. Each tube for sugar has 2 ml blood, for salt has 3 ml blood, and for distilled water has 4 ml blood. We mixed aforementioned ten varying concentrations of each analyte into each heparin tube. We put each sample in centrifuge machine at 800 rpm for 4 min to prepare platelet rich plasma. Then, we put one drop of PRP from each sample on each slide and examined it after covering each slide with cover slip under dark field microscope at 40×. Whole experiment was conducted at room temperature. PRP of each sample of each analyte is shown in Fig. 11.1.

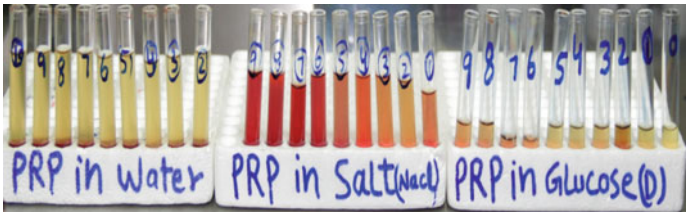

Fig. 11.1 Showing platelet rich plasma of each analyte

11.3 Effects of Glucose on Blood Cells

Glucose [$C_6H_{12}O_6$] has 180 molecular weight and has 180 g/L in one mole. It is one of the carbohydrates which are the best and vital source of energy. All sources of glucose are not good for health such as soft drinks and foods, although they are energy rich [12]. There exist four categories of sugar HFCS (corn starch), sucrose (table sugar), glucose (sugar in blood), and fructose (sugar in fruits). Adenosine triphosphate (ATP) which is the energy and is released when glucose is glycolysed [13]. Glucose level without and with meal is round about 70–99 mg/dL and 140 mg/dL, respectively [14]. Its level in blood constitutes either hyperglycemia (glucose more than normal level) or hypoglycemia (glucose less than normal level) [15]. The efficiency of platelet cells is increased due to resistance or less production of insulin either by hyperglycemia or by hypertriglyceridemia by the processes of glycation or osmotic burst/lyses of cells during abnormal metabolic process. Platelet cells release excess calcium by reducing the production of nitric oxide and disable the cellular endothelial by the processes like inflammation and oxidative stress. Thus, in diabetes platelet cells become more effective [16, 17].

11.3.1 Results

Two-dimensional photograph of glucose analyte under light microscope of each sample for ten varying concentrations (0–450 mM) is shown in Fig. 11.2a–j

11.3.2 Regions of Interest of WBCs for Sugar Analyte

Two-dimensional photographs of glucose analyte for platelet cells under dark field microscopy for each sample range (0–450 mM) are shown in Fig. 11.3a–j.

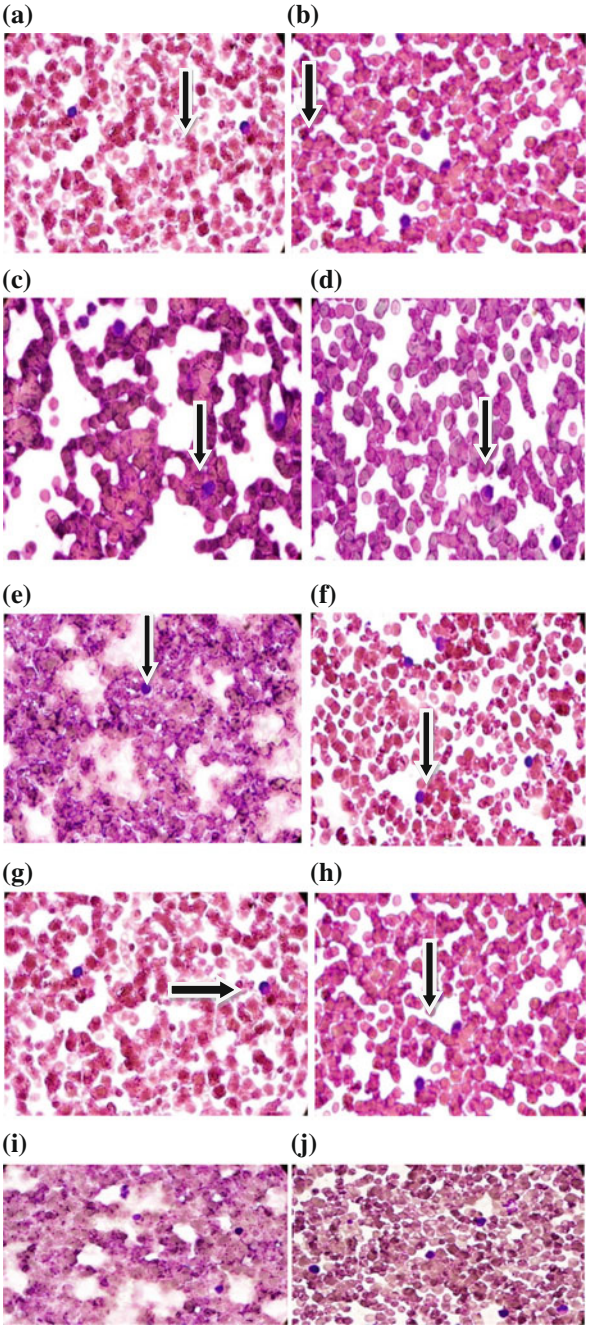

Fig. 11.2 2-D images of whole blood phantom prepared under white light microscope with 2 mL blood in heparin tube for **a** 0 mM (original sample) concentration of glucose, **b** 50 mM (18 mg) concentration of glucose, **c** 100 mM (36 mg) concentration of glucose, **d** 150 mM (54 mg) concentration of glucose, **e** 200 mM (72 mg) concentration of glucose, **f** 250 mM (90 mg) concentration of glucose, **g** 300 mM (108 mg) concentration of glucose, **h** 350 mM (126 mg) concentration of glucose, **i** 400 mM (144 mg) concentration of glucose and, **j** 450 mM (162 mg) concentration of glucose

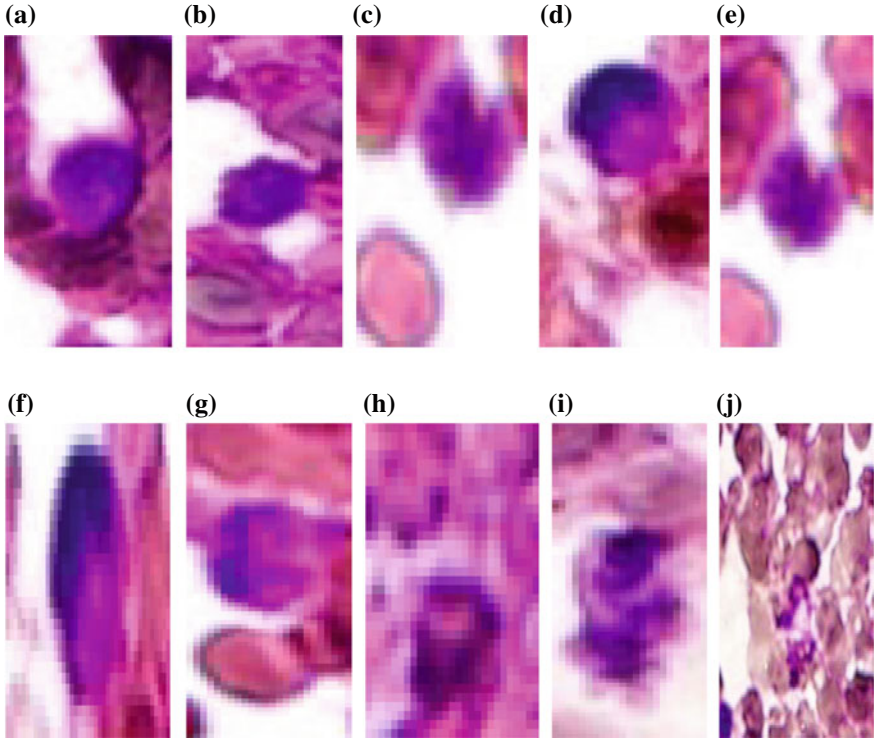

Fig. 11.3 Regions of interests (ROIs) for WBCs under sugar analyte concentrations 0–450 mM (**a**–**j**)

11.3.3 Statistical Comparison Under Glucose [$C_6H_{12}O_6$]

We admixed aforementioned ten different concentrations of sugar from 0 up to 450 mM with a step size of 50 mM into 2 ml blood, performed CBC of each sample with the help of celltac α hematological analyzer, and noted the blood components and parameters as given in Table 11.1.

11.3.4 Discussions and Conclusive Remarks

Sugar is a dietary part but its abnormal use creates metabolic disorders which further disturb many of the biological systems. Here, we focus on blood cells leukocytes and thrombocytes. Under increasing concentration of sugar, size of WBCs increases from normal size due to diffusion of sugar into cells and finally they burst/lyse as shown in Fig. 11.2a–j. Size of platelet cells starts increasing for 0–250 mM and cells clump with each other i.e. Rouleaux formation then further

Table 11.1 Change in parameters and cells count relative to glucose [$C_6H_{12}O_6$] concentrations

Sr. No.	Sugar concentration (mM)	No. of WBCs (10^3/μL)	No. of platelet cells (10^3/μL)	No. of RBCs (10^6/μL)	HGB (g/dL)	RDW (%)	PDW (%)	MCV (fL)	MPV (fL)	HCT (%)	PCT (%)
1	0	6.3	289	2.88 L	7.3 L	12.6	17.0	73.3 L	8.5	21.1 L	0.25
2	50	5.9	284	2.88 L	7.4 L	12.4	17.4	73.3 L	8.1	21.1 L	0.23
3	100	5.0	253	2.90 L	7.4 L	13.0	17.9	73.4 L	8.0	21.3 L	0.20
4	150	4.4	259	2.86 L	7.2 L	12.8	17.2	74.1 L	7.7	21.2 L	0.20
5	200	4.6	244	2.85 L	7.2 L	12.9	17.3	74.0 L	7.8	21.1 L	0.19
6	250	4.4	256	2.81 L	7.1 L	13.0	17.3	73.7 L	7.9	20.7 L	0.20
7	300	4.3	240	2.86 L	7.1 L	12.6	18.3H	74.1 L	7.6	21.2 L	0.18
8	350	4.3	247	2.79 L	7.1 L	13.1	17.3	74.2 L	7.6	20.7 L	0.19
9	400	4.4	290	2.75 L	7.0 L	12.7	17.4	73.5 L	8.2	20.2 L	0.24
10	450	4.5	307	2.79 L	7.0 L	13.1	17.9	74.2 L	8.0	20.7 L	0.25

addition of glucose from 300 to 450 mM they shrink in size as shown in Fig. 11.4. Shape of WBCs is also effected by hyperglycemia; the cells change from regular spherical to elongated up to 300 Mm and then change into elliptical shape at 450 mM as shown in Figs. 11.1, 11.2, while shape of platelet cells becomes elliptical up to 250 mM and then starts becoming normal like a plate above 300 mM as shown in Fig. 11.5. Sugar has same chemical structure like vitamin C. Vitamin C boosts up immune system by helping the leukocytes to fight against pathogens, while sugar weakens the defensive system. So, hyperglycemia not only weakens the defensive system by reducing the activity of WBCs but also count of leukocytes by destroying them. Thrombocytes count from 0 to 300 mM decreases gradually because insulin controls the sugar level, but as the sugar level increases above 300 mM then platelet cells start increasing as shown in Fig. 11.6. Thus, diabetes can cause deficiency of insulin, disorders of cellular walls, metabolic disorders, and inflammation, but platelet activity is increased by diabetes [21]. Hence, there is a suggestion about treatment of dengue with diabetes. RBCs also decrease in numbers under hyperglycemia due to bursting/lyses as shown in Fig. 11.6. HGB is a red pigment present in blood which plays a vital role to carry oxygen to different parts of body and gives red color to blood. HGB level gradually decreases with increasing concentration of glucose as shown in Fig. 11.7. HCT or PCV (packed cell volume) or EVF (erythrocytes volume fraction) is the volume percentage of RBCs. Its abnormal value is life threatening, and a low HCT or EVF value is noticed against increasing concentration of sugar as shown in Fig. 11.7. Low HCT results in anemia and leukemia. MCV is the measure of average volume of RBCs and is helpful in classification of type of anemia. MCV shows increasing trend under hyperglycemia as shown in Fig. 11.7 and thus showing trend from normocytic anemia to macrocytic anemia. RDW is the measure of range of variations of RBCs. RDW shows an increasing trend with increasing concentration of sugar as shown in Fig. 11.7. High value of RDW also causes anemia. MPV is the measure of average size of platelet cells and is useful to predict the destruction or production of platelet cells. High MPV results in destruction of platelet cells. Figure 11.7 shows decreasing trend of MPV and thus indicates production of cells. PDW is used to express the variations in size of platelet cells. PDW increases with increasing concentration of sugar. MPV and PDW are related with each other and are generally direct in relation but here is opposite condition as shown in Fig. 11.7. Low MPV with high PDW results in anemia. PCT is a source to measure quantitative disorders/abnormalities of platelet cells. PCT shows thrombocytopenia up to 350 mM and then thrombocytosis up to 450 mM as shown in Fig. 11.7. HGB, RDW, HCT, and MCV are the parameters related to RBCs, while MPV, PDW, and PCT are related with platelet cells.

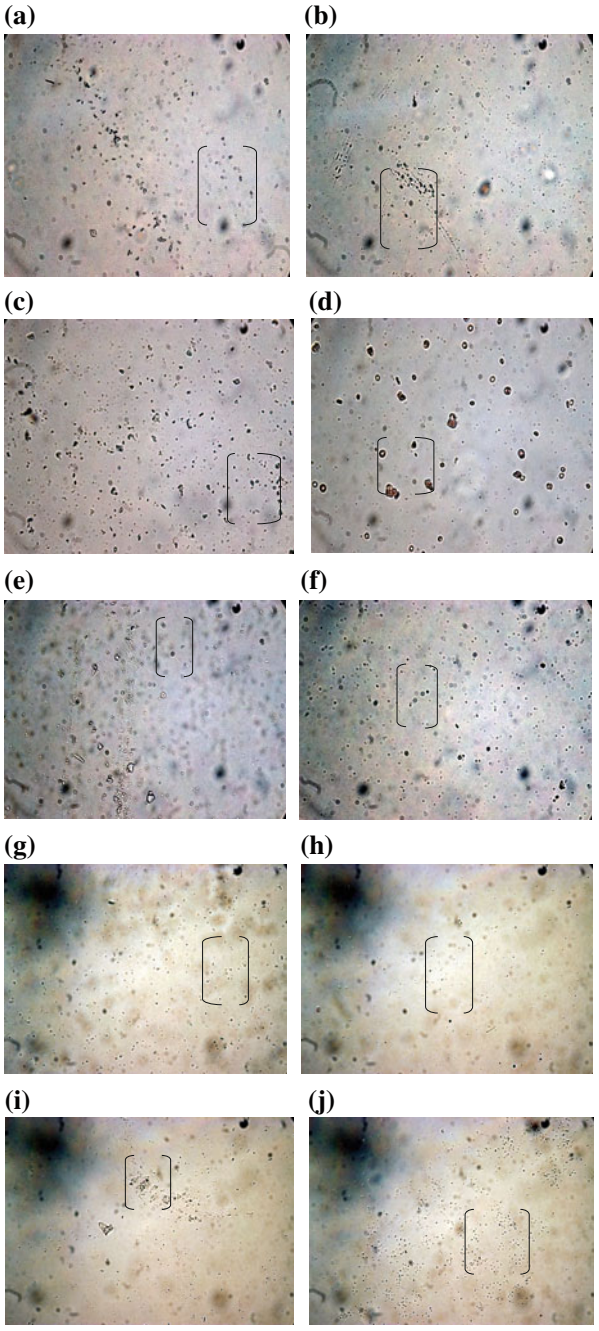

Fig. 11.4 Dark field microscopic 2-D images of PRP phantom prepared with 2 mL in EDTA tube for **a** 0 mM concentration of glucose, **b** 50 mM (18 mg) concentration of glucose, **c** 100 mM (36 mg) concentration of glucose, **d** 150 mM (54 mg) concentration of glucose, **e** 200 mM (72 mg) concentration of, glucose, **f** 250 mM (90 mg) concentration of glucose, **g** 300 mM (108 mg) concentration of glucose, **h** 350 mM (126 mg) concentration of glucose, **i** 400 mM (144 mg) concentration of glucose and, **j** 450 mM (162 mg) concentration of glucose

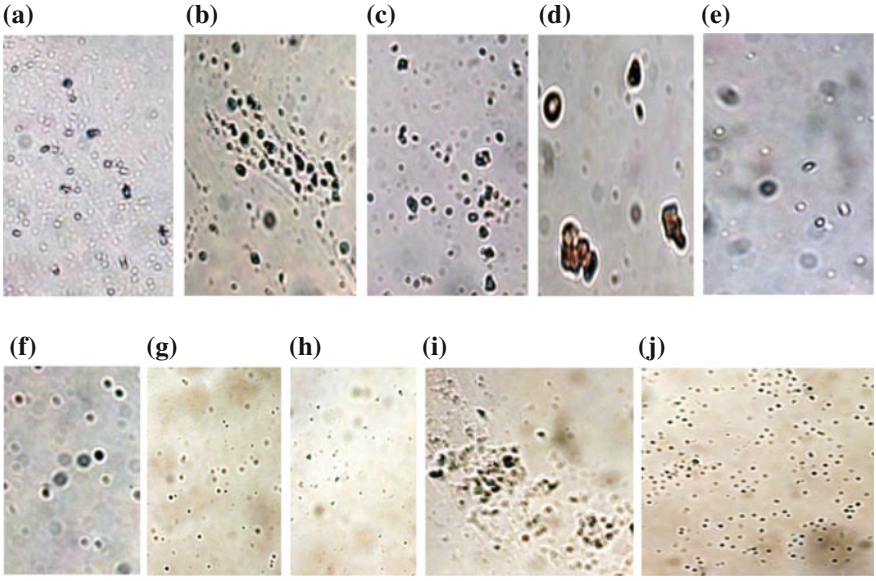

Fig. 11.5 ROIs of platelet cells for sugar analyte's concentrations (0–450 mM)

11.4 Effects of Salt (NaCl) on WBCs and Platelet Cells

Salt maintains homeostasis in body due to its vital dietary part of our routine. Its normal value is between 135 and 145 mM/L in the human body. Depending upon concentration, it has two conditions, less than normal value and higher than normal value are termed as hyponatremia and hypernatremia respectively [18–20]. NaCl is its chemical formula with 58.5 as its molecular weight. Blood pressure is associated with concentration of salt. NaCl is also used in medicine, agriculture, as cleansing agent, in food industry, and in pure form used as optical agent [21]. Hypernatremia results in production of protein-like interleukin which in turn produces large amount of helper cells which damages the defense system by destroying healthy tissues (Table 11.2). This situation is termed as autoimmunity. So, hypernatremia not only results in high blood pressure but also weakens the immune system [22, 23]. NaCl is very vital dietary part and causes high blood pressure due to its high usage. Many disorders are caused by its accumulation in blood capillaries like resistance against activities of arteries, increase in renal function, increase in mass of left ventricle, increase in number of strokes and increase in the stiffness, and disorders in cardiovascular system [19]. During hypernatremia, the platelet cells function is increased due to accumulation of (Na) [24, 25]. Hypernatremia shows Rouleaux formation (grouping or colonies formation) of platelet cells due to high blood pressure with and without family history. Molecular weight of sodium chloride is 58.5. Its chemical formula is NaCl.

Table 11.2 Change in blood parameters and cells count relative to salt (NaCl) concentrations

Sr. No.	Salt (NaCl) concentration (mM)	No. of WBCs (10^3/μL)	No. of platelet cells (10^3/μL)	No. of RBCs (10^6/μL)	HGB (g/dL)	RDW (%)	PDW (%)	MCV (fL)	MPV (fL)	HCT (%)	PCT (%)
1	0	6.3	289	2.88 L	7.3 L	12.6	17.0	73.3 L	8.5	21.1 L	0.25
2	50	4.9	325	2.73 L	6.9 L	13.2	17.4	74.4 L	8.1	20.3 L	0.26
3	100	4.5	341	2.74 L	7.0 L	13.0	17.9	74.1 L	7.8	20.3 L	0.27
4	150	4.0	327	2.80 L	7.0 L	12.5	18.0	73.9 L	8.3	20.7 L	0.27
5	200	3.8	327	2.94 L	7.4 L	12.6	17.9	74.5 L	7.9	21.9 L	0.26
6	250	3.6	306	2.92 L	7.4 L	13.2	17.2	74.7 L	7.7	21.8 L	0.24
7	300	3.9	321	3.08 L	7.8 L	12.7	17.3	74.0 L	7.7	22.8 L	0.25
8	350	3.6	318	3.02 L	7.8 L	12.8	17.8	75.2 L	7.8	22.7 L	0.25
9	400	2.2	309	2.20 L	5.5 L	13.6	18.3H	75.5 L	7.7	22.5 L	0.24
10	450	3.5	321	2.98 L	7.5 L	13.8	17.2	76.2 L	7.6	22.7 L	0.24

11.4.1 Materials and Methods

For the effects of salt on WBCs and Platelet cells, same method has been adopted as given in Sect. 1.3.1 with 3 ml blood for each phantom.

11.4.2 Results

Two dimensional photographs of WBCs of each phantom for aforementioned ten varying concentration of NaCl from 0 to 450 mM under microscope with bright field at 100X are given in Fig. 11.8.

11.4.3 Regions of Interest for WBCs Under Salt

Regions of Interest of WBCs (ROI) of each phantom for aforementioned ten varying concentration of NaCl from 0 to 450 mM under microscope with dark field at 40X are given in Fig. 11.9.

11.4.4 Regions of Interest for Platelet Cells Under Salt

ROIs of platelet cells of each phantom for aforementioned ten varying concentration of NaCl from 0 to 450 mM are given in Fig. 11.10

11.5 Discussions

Hypernatremia causes high blood pressure in general. WBCs are more affected than RBCs by NaCl because they have membrane and nucleus. Under hypernatremia water moves out of blood vessels and causes dehydration. When concentration of salt is less as compared to normal value then water will move into blood vessels. This type of changes brings renal disorders [26]. WBCs under hypernatremia concise or reduce in size and bring changes in shape from round to elliptical as shown in Fig. 11.12a–j, while platelet cells also show size shrinkage and shape deformation due to Rouleaux formations with increasing concentration of salt as shown in Fig. 11.11a–j. Under hypernatremia, count of WBCs goes high which is harmful because these cells start damaging the healthy tissues and the condition is known as autoimmunity like sclerosis (hardening of tissues) diabetes type-1 and [18, 27]. WBCs count goes on decreasing up to 400 Mm and then above 400 Mm goes on increasing as shown in Fig. 11.8. Platelet cells count is not linear factor due to Rouleaux formation for some middle concentration samples, but graphically under hypernatremia number of platelet cells increases generally as shown in Fig. 11.8. HGB is a red pigment present in

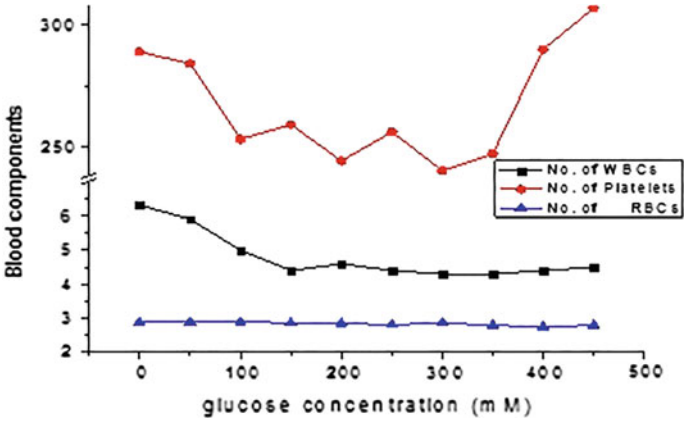

Fig. 11.6 Comparison of sugar concentrations and blood cells

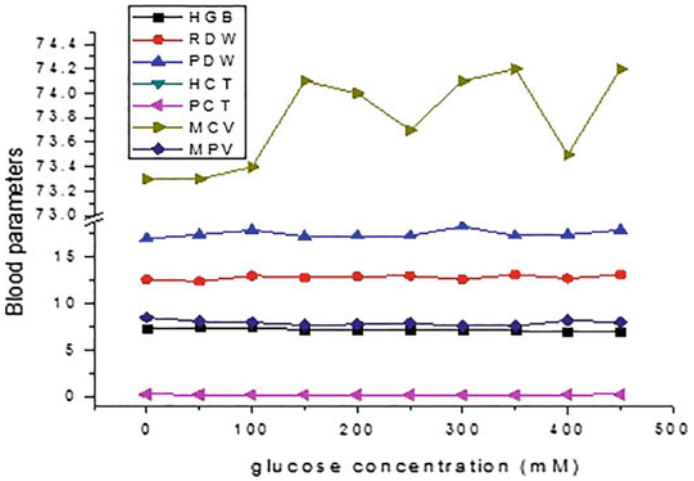

Fig. 11.7 Comparison of sugar concentrations and blood parameters

blood which plays a vital role to carry oxygen to different parts of body and gives red color to blood. HGB level is less than normal value up to 150 mM and then starts increasing with increasing concentration of NaCl up to 450 mM as shown in Fig. 11.9. So it can be suggested that high dose of salt can be used to improve HGB level in blood. HCT or PCV (packed cell volume) or EVF (erythrocytes volume fraction) is the volume percentage of RBCs. Low HCT results in anemia and leukemia. Its abnormal value is life threatening, and a high HCT or EVF value is noticed against increasing concentration of salt as shown in Fig. 11.13. MCV is the measure of average volume of RBCs and is helpful in classification of type of anemia. MCV shows increasing trend under hypernatremia as shown in Fig. 11.9 and thus showing

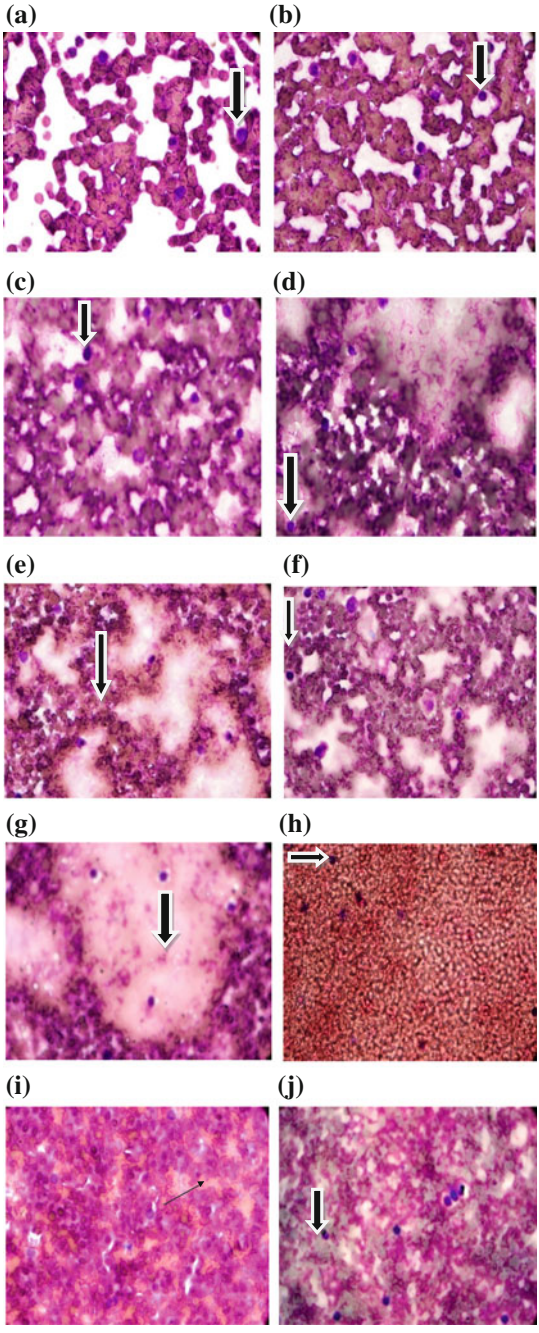

Fig. 11.8 a–j Showing photograph of WBCs of each phantom for ten varying concentrations of salt ranging from 0 to 450 mM with step size of 50 mM, respectively, under microscope at 100X

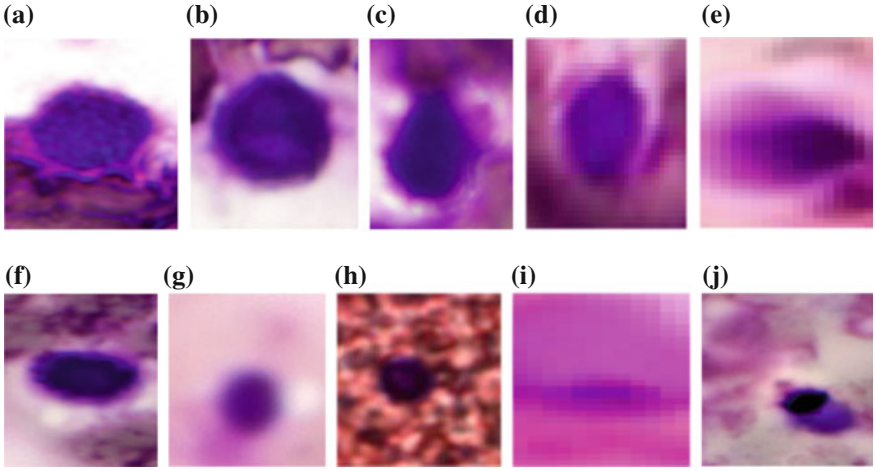

Fig. 11.9 ROIs of WBCs under salt concentrations (0–450 mM)

trend from normocytic anemia to macrocytic anemia. RDW is the measure of range of variations of RBCs. RDW shows an increasing trend with increasing concentration of salt as shown in Fig. 11.9. High value of RDW also causes anemia. MPV is the measure of average size of platelet cells and is useful to predict the destruction or production of platelet cells. High MPV results in destruction of platelet cells. Figure 11.9 shows decreasing trend of MPV and thus indicates production of cells. Thus, it can be suggested that high usage of salt is beneficial to overcome low platelet count in dengue patients. PDW is used to express the variations in size of platelet cells. PDW increases with increasing concentration of sugar. MPV and PDW are related with each other and are generally direct in relation but here is opposite condition as shown in Fig. 11.9. Low MPV with high PDW results in anemia. PCT is a source to measure quantitative disorders/abnormalities of platelet cells. PCT shows trend of thrombocytopenia up to 200 mM and then thrombocytosis up to 450 mM as shown in Fig. 11.9. HGB, RDW, HCT, and MCV are the parameters related to RBCs, while MPV, PDW, and PCT are related with platelet cells.

11.6 Effects of Distilled Water on Blood Cells

Water can move inside and outside of cells due to osmosis and diffusion because membranes of cells are made up of permeable proteins. Water due to its movement within the cells forms three types of solutions: hypertonic solution, hypotonic solution, and isotonic solution [18]. **Hypertonic solutions:** When the concentration of water is greater inside the cell than outside is called hypertonic solution. Under

hypertonic solution water will move from inside to outside and cell will shrink and unable to perform natural process of cell division [18]. **Hypotonic solutions:** When the concentration of water is greater outside the cell than inside is called hypotonic solution. Under hypotonic solution, cell will swell and finally burst/lyse [18]. **Isotonic solutions:** If concentration of water inside and outside of cells is same then solution is termed as isotonic solution. Under isotonic solution, cells remain neutral [18].

11.6.1 Results

Two dimensional photographs of WBCs of each phantom for aforementioned ten varying concentration of water from 0 to 450 mM under microscope with bright field at 100X are shown in Fig. 11.14.

11.6.2 Regions of Interest for WBCs Under Water

2-D images of dark field microscopy of each phantom for ten different concentrations (0–450 mM) of water analyte with step size 50 mM are shown in Fig. 11.15a–j

11.6.3 Regions of Interest for Platelet Cells Under Water

ROIs of platelet cells of each phantom for aforementioned ten varying concentration of H_2O from 0 to 450 mM are given in fig. 11.16.

11.7 Statistical Comparison of Blood Components and Parameters Under Pure Water (H_2O)

We admixed ten different concentrations of water from 0 up to 450 mM with a step size of 50 mM into 4 ml blood and performed CBC of each sample with the help of celltac α hematological analyzer and noted the blood components and parameters as given in Table 11.3 and have been plotted in Figs. 11.17 and 11.18.

Table 11.3 Change in parameters and cells count relative to pure water (H_2O) concentrations

Sr. No.	Water concentration (µl)	No. of WBCs (10^3/µL)	No. of platelet cells (10^3/µL)	No. of RBCs (10^6/µL)	HGB (g/dL)	RDW (%)	PDW (%)	MCV (fL)	MPV (fL)	HCT (%)	PCT (%)
1	0	6.3	289	2.88 L	7.3L	12.6	17.0	73.3 L	8.5	21.1 L	0.25
2	50	5.7	331	2.98 L	7.6L	12.5	17.9	73.2 L	8.0	21.8 L	0.26
3	100	5.7	354	2.92 L	7.5L	12.5	17.9	72.6 L	8.2	21.2 L	0.29
4	150	5.5	333	2.80 L	7.0 L	12.5	18.2	73.9 L	8.3	20.7 L	0.27
5	200	5.7	349	2.94 L	7.4 L	12.6	17.9	74.5 L	7.9	21.9 L	0.26
6	250	5.7	349	2.92 L	7.4 L	13.2	17.2	74.7 L	7.7	21.8 L	0.24
7	300	5.7	333	3.08 L	7.8 L	12.7	17.3	74.0 L	7.7	22.8 L	0.25
8	350	5.8	375	3.02 L	7.8 L	12.8	17.8	75.2 L	7.8	22.7 L	0.25
9	400	5.7	345	2.02 L	5.5 L	13.6	18.3H	75.3 L	7.7	16.7 L	0.24
10	450	5.6	344	2.98 L	7.5 L	13.8	17.2	76.2 L	7.6	22.7 L	0.24

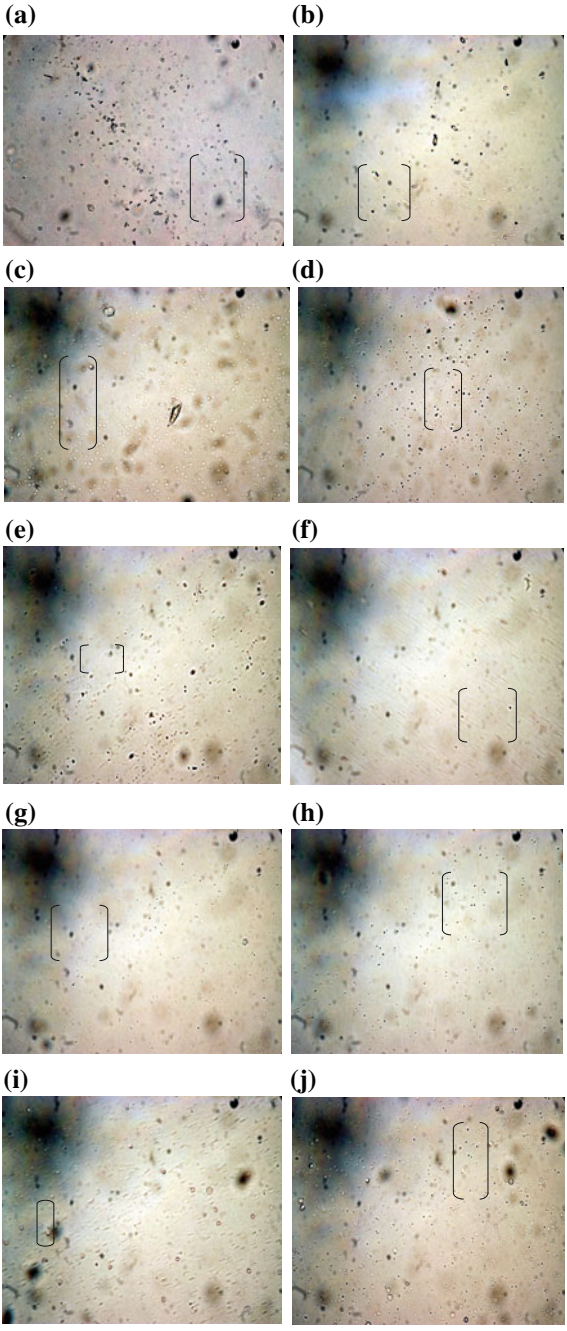

Fig. 11.10 a–j Showing photograph of platelet cells of each phantom for ten varying concentrations of salt ranging from 0 to 450 mM with step size of 50 mM, respectively, under dark field microscope at 40X

11.8 Results and Discussions

Under hypotonic solution of distilled water, the size of WBCs increases with increasing concentration of pure water. Leukocytes get swell and burst/lyse as shown in Fig. 11.15. Like leukocytes, platelet cells also grow in size with increasing concentration of distilled water as shown in Fig. 11.16 and region of interest for platelet cells under distilled water. Shape of WBCs also changes gradually from round to elliptical due to swelling by osmosis processes as shown in Fig. 11.15 and region of interest for leukocytes under distilled water. Thrombocytes also show same changes in shape as shown by leukocytes they become elongated from plate shape and finally elliptical as shown in Fig. 11.16 and region of interest for platelet cells under distilled water. Leukocytes count under hypotonic solution decreases due to bursting of cells as shown in Fig. 11.17. Platelet cells show opposite count; they increase in number up to 350 mM and then decrease as shown in Fig. 11.17. HGB is a red pigment present in blood which plays a vital role to carry oxygen to different parts of body and gives red color to blood. HGB level starts increasing from 50 mM, remains high up to 350 mM, and then starts decreasing as shown in Fig. 11.18. So it can be suggested that at very high concentration of pure water HGB level in blood becomes low and cannot perform properly [28]. HCT or PCV (packed cell volume) or EVF (erythrocytes volume fraction) is the volume percentage of RBCs. Low HCT results in anemia and leukemia. Its abnormal value is life threatening; a high HCT or EVF value is noticed against increasing concentration of water as shown in Fig. 11.18. MCV is the measure of average volume of RBCs and is helpful in classification of type of anemia. MCV shows increasing trend under hypernatremia as shown in Fig. 11.18 and thus showing trend from normocytic anemia to macrocytic anemia. RDW is the

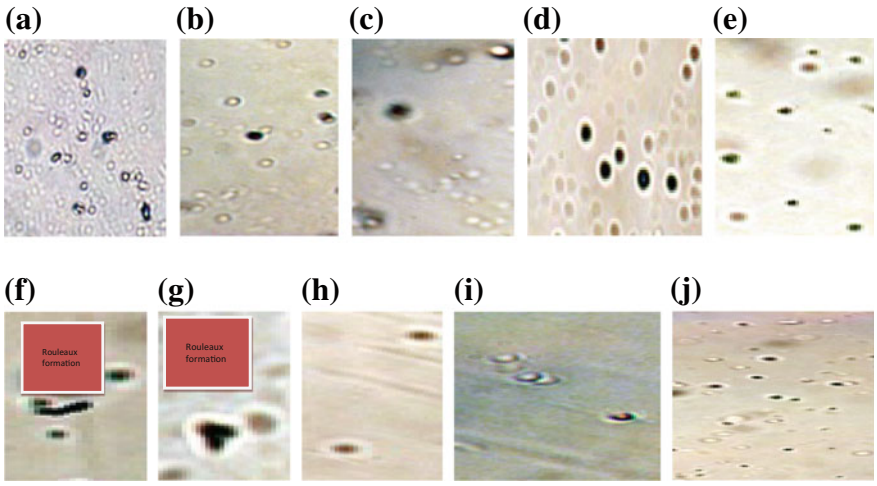

Fig. 11.11 ROIs for platelet cells under salt concentrations (0–450 mM)

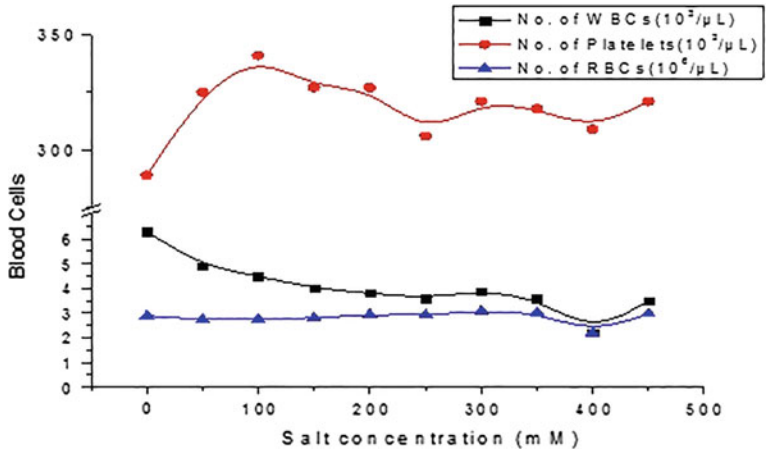

Fig. 11.12 Salt concentrations versus blood cells

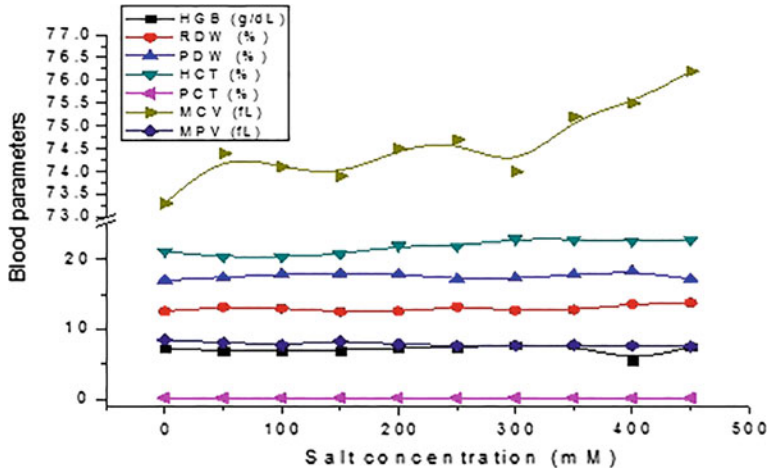

Fig. 11.13 Salt concentrations versus blood parameters

measure of range of variations of RBCs. RDW shows decreasing trend up to 150 mM, and with further increasing concentration of water it increases up to 450 mM as shown in Fig. 11.18. High value of RDW also causes anemia [29]. MPV is the measure of average size of platelet cells and is useful to predict the destruction or production of platelet cells. High MPV results in destruction of platelet cells. Figure 11.18 shows decreasing trend of MPV and thus indicates production of cells. Thus, it can be suggested that high usage of water is beneficial to overcome low platelet count in dengue patients. PDW is used to express the variations in size of platelet cells. PDW increases with increasing concentration of

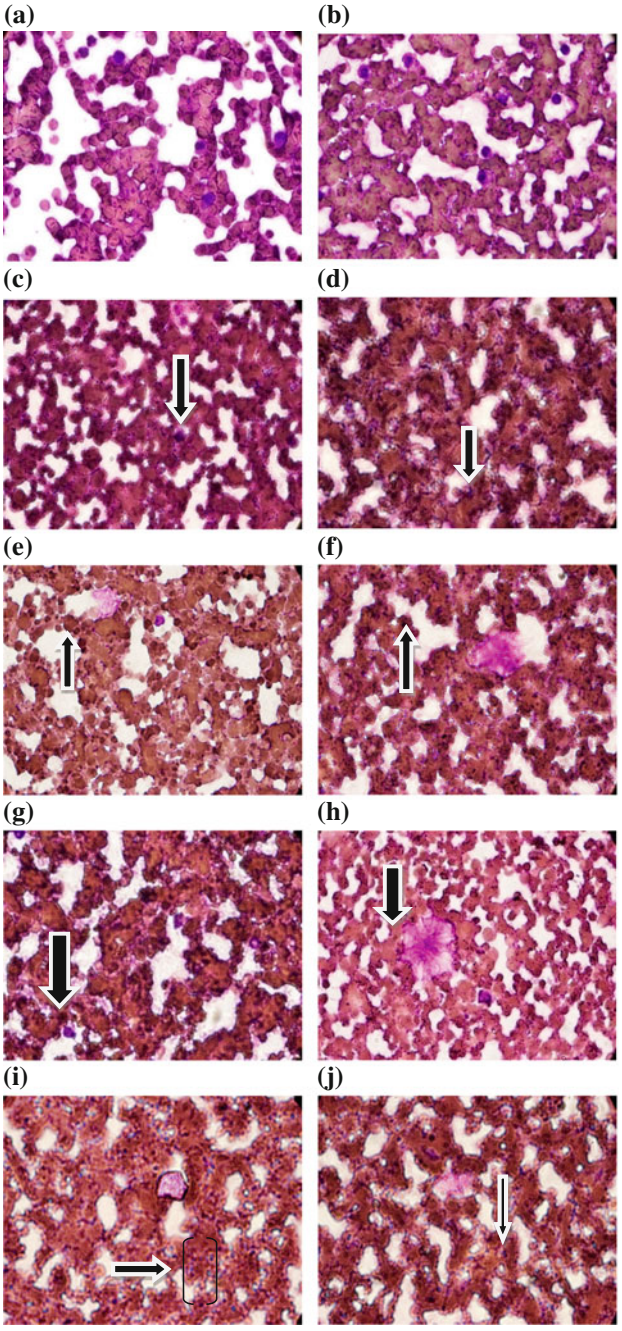

Fig. 11.14 a–j Two-dimensional images for each sample of WBCs ranging from 0 up to 450 mM using white field microscopy under transmission mode at 100X

water up to 400 mM. MPV and PDW are related with each other and are generally direct in relation but here is opposite condition as shown in Fig. 11.18. Low MPV with high PDW results in anemia. PCT is a source to measure quantitative disorders/abnormalities of platelet cells. PCT shows almost no change under increasing concentration of pure water as shown in Fig. 11.18 [30]. HGB, RDW, HCT, and MCV are the parameters related to RBCs, while MPV, PDW, and PCT are related with platelet cells (Fig 11.18).

11.9 Summary

Blood is composed of blood cells and plasma with many parameters. Blood in our body performs many important functions like supply of oxygen and nutrients, messenger functions, removal of waste materials, immunological functions, coagulation functions, regulation of body temperature, and pH hydraulic functions. These above given functions are disturbed by the abnormal use of these analytes. In this, we have given a detailed and depth knowledge about shape, size, and cells count of both leukocytes and thrombocytes. Under hypercondition of glucose, salt and distilled water shape of both cells changes almost from round to elliptical. Size of cells increases under hypercondition of glucose and distilled water which results

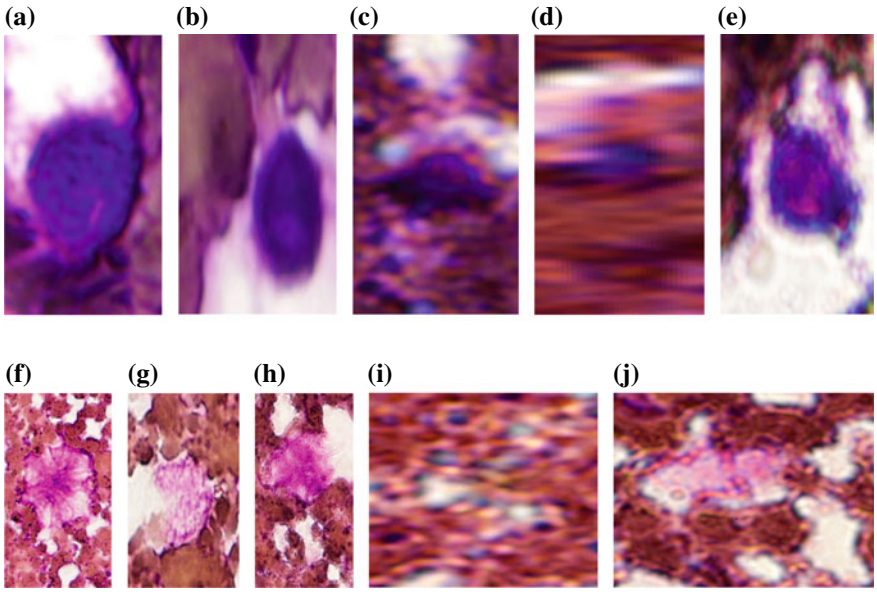

Fig. 11.15 ROIs for WBCs under pure water concentrations (0–450 mM) **a–j** Two-dimensional images of each sample range plateleting from 0 up to 450 mM showing the changes in shape and size of cells using dark field microscopy under transmission mode at ×40

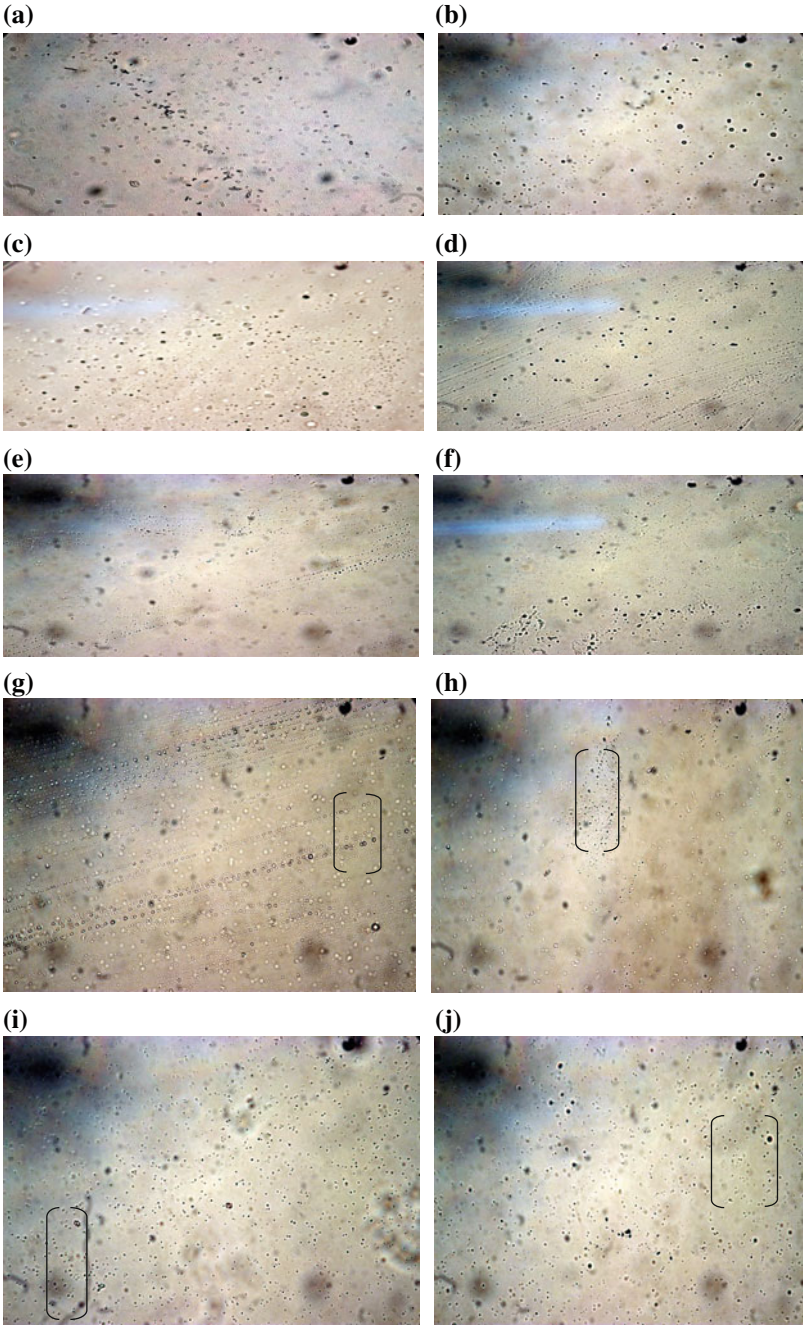

Fig. 11.15 (continued)

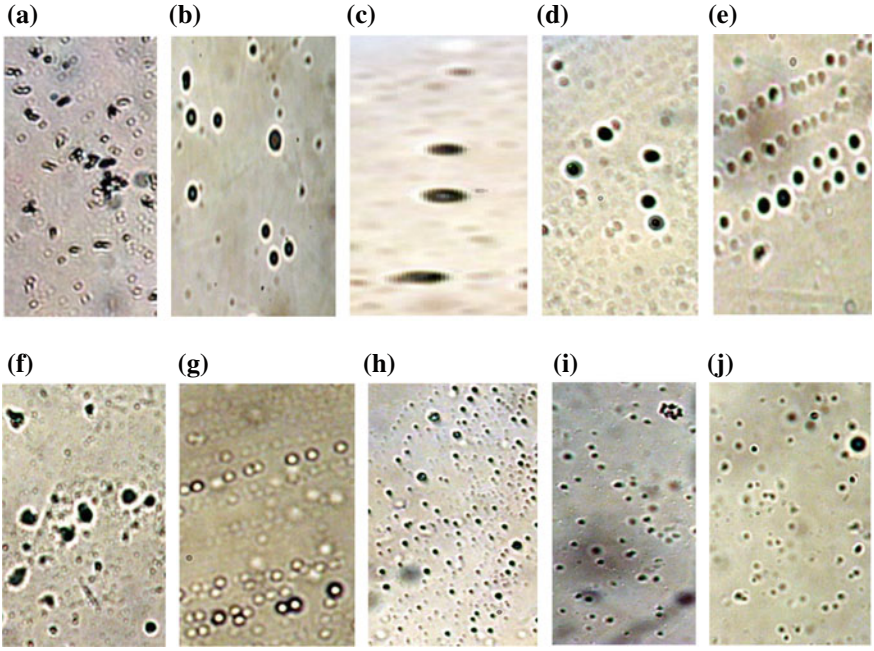

Fig. 11.16 ROIs for platelet cells under water concentrations (0–450 mM)

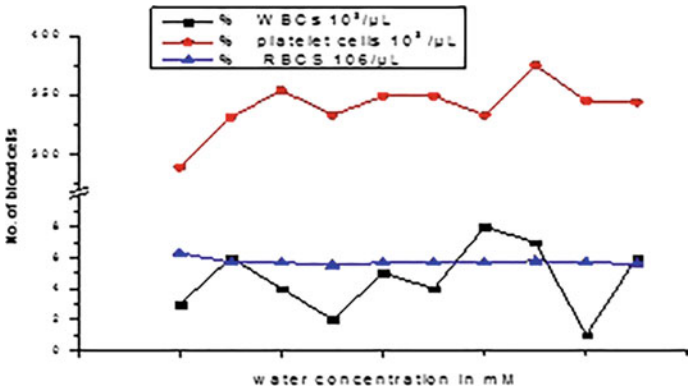

Fig. 11.17 Comparison of pure water concentrations and blood cell

lyses of cells, while leukocytes concise. Cells count of leukocytes under hypercondition of three analytes decreases for both glucose and distilled water, while under salt analyte they increase in number above 400 mM and cause autoimmune diseases. Count of thrombocytes under hypercondition of glucose and salt increases very slowly but under distilled water their count goes on increasing very sharply. HGB a red pigment in blood increases with increasing concentration of salt and

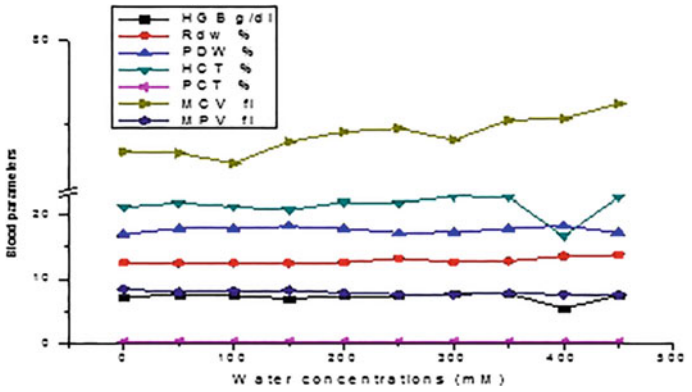

Fig. 11.18 Comparison of salt concentrations versus blood parameters and blood cells

distilled water but for sugar it decreases. HCT or PCV (packed cell volume) or EVF (erythrocytes volume fraction) decreases for sugar and increases for salt and distilled water little bit with increasing concentration. MCV in blood increases with increasing concentration of salt and distilled water but for sugar it decreases. RDW and PDW increase with increasing concentration of all three analytes. MPV decreases with increasing concentration of all three analytes. PCT which measures quantitative abnormalities of platelet cells almost remains same under increasing concentration of three analytes with few fluctuations in its values. All parameters almost have same value and same trend for each concentration. Thus, abnormal use of any of three analyte is toxic to many physiological, biochemical and metabolic processes, biological systems, and organs like immune system, respiratory system, blood circulatory system, lymphatic system, enzymes system and failures of organs like liver and heart.

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