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**JOURNAL OF INDUS MEDICAL COLLEGE (JIMC)**

# EDITORIAL BOARD

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EDITORIAL

# EXAGGERATED EXERCISE, BLOOD PRESSURE AND LOWER LEVEL OF INFLAMMATION

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An abnormal rise in systolic blood pressure (BP) during exercise is observed in a portion of individuals with normal BP at rest. Such response has been associated with an increased risk for future hypertension and cardiovascular events. [1-2] The factors that influence this exaggerated rise in BP during exercise are not established. However, impaired endothelial function in the setting of excessive elevations in exercise BP has been reported recently. [3]

Endothelium, the innermost layer of all blood vessels, is not simply an inert barrier that separates blood from tissue but is a dynamic organ that plays a vital role in vasomotion and, consequently, blood flow regulation, coagulation, fibrinolysis, and inflammation. Intact endothelium is essential for maintaining normal vascular tone and relaxation. This is accomplished by a delicate balance between endothelium - derived vasodilating, mainly nitric oxide, and vasoconstricting agents. [4] During exercise, the increased blood flow increases the mechanical stress in the vessels (shear stress). This provides the stimulus for the intact endothelium to accelerate the release of nitric oxide, resulting in vasodilatation and a reduction in peripheral resistance. However, under the same exercise conditions, but with endothelial integrity compromised, nitric oxide bioavailability in response to shear stress (exercise) is reduced, resulting in an impaired vasodilatation and increased peripheral resistance. [5] Therefore, it is reasonable to assume that the excessive BP elevation observed during exercise may be caused by the impaired vasodilatation resulting from endothelial dysfunction. An association between an exaggerated BP response during exercise and impaired endothelial function has been reported. [3] Acute systemic inflammation has also been reported to profoundly impair endothelium - dependent vasodilatation in humans. [6]

In Journal of Cardiopulmonary Rehabilitation, Sae Young Jay and colleagues, [7] proposed that an exaggerated BP response may be the result of impaired endothelial function that can be detected by increased levels of low-grade inflammation markers. For this, they assessed exercise BP, C-reactive protein (CRP), and white blood cell (WBC) in 43 individuals with normal resting BP but an abnormal BP response at peak exercise. Another 42 individuals with normal BP

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at rest and during exercise served as controls. The investigators reported that the WBC count in those who exhibited an exaggerated BP response during exercise was significantly higher (approximately 15%) than that of the group with normal BP response. CRP levels were similar between the 2 groups and only a trend toward a positive association between CRP levels and WBC count was observed. They concluded that these findings suggest that low-grade inflammation may be associated with an exaggerated BP response at peak exercise.

As mentioned by the authors, increased WBC count has been associated with the development of hypertension (and perhaps inflammation). However, the lack of difference in CRP levels between the 2 groups in this study is disappointing and detracts from this conclusion. Because CRP is a much more sensitive and specific indicator of systemic inflammation than is the WBC count, one may ask why CRP levels were not increased in the group exhibiting an exaggerated BP response if inflammatory processes were involved. In addition, it is important to point out that the WBC count was very much within the reference range ($6.1x10^9$/L Vs $5.2x10^9$/L) for those with an exaggerated and normal BP response, respectively.

It would also be of interest to know if blood was drawn before or after the exercise test. If blood was drawn before exercise, the association between WBC count and inflammation is strengthened. However, if blood was drawn after the completion of the exercise test, it is possible that the higher WBC count was an acute response to the stressor induced by higher exercise BP and not the result of inflammation. Unfortunately, these data are not available to address this question. Despite these limitations, this study helps to generate some interesting prospects. The prospect that an exaggerated BP response during exercise may be an indicator for low-grade inflammation and impaired endothelial function is certainly enticing and should be explored further. Indeed, the additional information provided by an exaggerated BP response during a graded exercise test can have important clinical applications. Because exercise testing is already widely used, a wealth of information on exercise BP response exists. The information can then be used to examine associations between exercise BP responses and inflammatory markers. For future patients undergoing exercise testing, such information can easily be obtained during exercise testing at no additional cost because additional equipment or procedures are not required. Exercise BP response can be used as a non-invasive and relatively inexpensive screening tool for those at risk for hypertension, left ventricular hypertrophy, or other cardiovascular events. Individuals with an exaggerated BP response can then be referred for further evaluation. Those with low-grade inflammation can be followed closely and treated for conditions or factors that foster the inflammatory process.

The therapeutic potential of exercise should also be considered. Current criteria recommend that antihypertensive therapy should be initiated for patients with confirmed hypertension, defined as systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg. Therefore, normotensive individuals who exhibit an exaggerated BP response during exercise are not likely to be treated with antihypertensive medication.

One study suggests that the exaggerated BP response during an acute bout of exercise can be attenuated by regularly performed, moderate-intensity exercises. They noted that moderate- and high-fit normotensive individuals exhibit lower BP at submaximal and maximum exercise levels, as well as 24-hour ambulatory BP compared with low-fit

individuals. Furthermore, low-fit individuals had higher left ventricular mass and greater likelihood of left ventricular hypertrophy than did their fit counterparts. [8] There was also a strong association between exercise BP and 24-hour BP, both strong predictors of left ventricular hypertrophy. In patients with severe hypertension, they reported that 16 weeks of low to moderate aerobic exercise resulted in significantly lower exercise BP at maximal and absolute submaximal exercise workloads. [9]

Collectively, these data suggest that exercise BP reflects the BP during routine daily activities. Regularly performed, moderate - intensity exercise is likely to attenuate an abnormal rise in daily BP and protect against associated health consequences. Is this accomplished by the restoration of endothelial function that is compromised by low-grade inflammation? We do not know yet. However, accumulating evidence suggests that increased physical activity is associated with significantly lower levels of inflammatory markers. [10]

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SHORT COMMUNICATION

# MOLECULAR DIAGNOSIS: A GAME CHANGER

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Successful care rendered by a healthcare professional to the patient relies on specific 'diagnosis' and 'treatment' of the ailment. For decades, first-line laboratory diagnostic applications such as Gram staining, staining of haematoxylin and eosin, full blood count, and other biochemical tests have been available. They have essential limitations, however. The area of diagnostics has been dynamically transformed by molecular diagnostics. It is a branch which provides a collection of techniques in the genetic code (genome) and protein expressed by the genes (proteome) to analyse biological markers. Its function in human diseases is commonly associated with the identification of mutations and pathogens. In laboratory medicine, the continuous availability of new methods and new applications has helped to make molecular diagnostics the fastest growing field.

We are trying to understand the ways in which diseases evolve in a person with ground breaking advances in molecular biology. Moreover, it also gives us an indication of the patient's disease resistance, immunity, and unique treatment. Hospitals, reference labs, and blood banks are commonly used for molecular diagnostics. Polymerase chain reaction (PCR), blotting techniques, fluorescent in situ hybridization (FISH), microarray, and, among others, mass spectrometry are the main technologies involved. These tests are commonly used in clinical applications, including infectious diseases, screening for genetic diseases, pharmacogenomics, oncology and typing of human leukocyte antigen. (1)

The most widely used process in major laboratories is PCR. It has revolutionised scientific study and medicine in the region. There are now approximately more than twelve updated forms of PCR with major advantages over each other. It plays a growing role in infectious

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diseases such as tuberculosis, HIV, hepatitis, and so on. While conventional laboratory tests can be used to diagnose infectious agents, PCR remains an excellent option as it is a quicker, more responsive and more accurate process. Similarly, in the area of histopathology, understanding the biology of lymphomas and leukaemia has also gained significance. Translocations are now being detected by rT-PCR in aggressive tumours such as sarcomas. In forensic sciences, genetics, and paternity testing, DNA profiling is also used. [2]

In contrast to the traditional method of karyotype analysis, the FISH technique has expanded the cytogenetics field, being faster and more precise. The identification of molecular markers and translocations in different cancers, including leukaemia, breast carcinoma, prostate cancer, cholangiocarcinoma, and melanoma, has become a critical tool in oncology. Literature, however, shows that developing countries have restricted use of FISH due to a lack of knowledge or unavailability of the process. [3] Microarray is another upcoming tool that, due to its cost and because it also requires professional hands to operate, is not yet routinely used in diagnostics. Yet, as accurate and responsive as other molecular diagnostic facilities, it claims to be. Molecular methods are also used in agricultural and industrial applications, in addition to the medical field. Moreover, gene therapy research is continuing and in the limelight. It is estimated that about 19,000 to 20,000 protein coding genes are found in the human genome. [4] In this age of personalised medicine, it is anticipated that a number of molecular methods will soon identify the entire human genome. The progress and new discoveries in the field of molecular biology have led to a paradigm change from standard diagnostic tests to the advancement of a variety of different diseases in diagnosis and treatment.

## CONCLUSION

Therefore, we conclude that knowledge of molecular biology and diagnostics for any medical practitioner has become important. Medical students can also acquire knowledge of molecular diagnostics in this age of modern medicine. In addition, in the medical education, emphasis should be on including molecular diagnostics.

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ORIGINAL ARTICLE

# ASSOCIATION OF RISK FACTORS FOR DIABETES MELLITUS AND SERUM ELECTROLYTES

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## ABSTRACT

**OBJECTIVE:** The purpose of the study was to examine the imbalance of selected serum electrolytes and the associated factors in diabetic patients.

**METHODOLOGY:**
This was a cross-sectional study, carried out in diabetic patients attending their follow-up appointments at the Polyclinic Hospital Islamabad. To include 155 patients with diabetes mellitus in the study, a convenience technique of sampling was used. A questionnaire was utilized to comprise all necessary information from each patient with diabetes mellitus. 5mL of venous whole blood was extracted from each participant and ion-selective electrode (ISE) device and automated chemistry analyzer were processed and tested for determination of serum electrolyte and serum glucose respectively. In order to determine the association and meaningful link between irregular electrolytes in serum and independent parameters, the model of Pearson's correlation coefficient and regression for multivariate logistic were performed respectively.

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**RESULTS:** In diabetic patients, an increased prevalence rate of disturbed electrolytes in the serum was prominent. The total prevalence rate was 45.80% (n=71/155), with hyponatraemia being the largest (46.47%), followed by hypochloraemia and hypercalcaemia (19.48% and 14.28% respectively. There were strong positive associations between type of medication, age and high body mass index (BMI) and elevated serum concentrations of sodium ($Na^+$) (r=0.712, P=0.003), potassium ($K^+$) (r=0.817, P=0.002) and chloride (Cl-) (r=0.518, P=0.003). In diabetic patients, risk factors for serum electrolyte disorders were statistically defined as being employed (AOR: 3.879, 95%, P-value: (0.044), treated with various drugs (AOR: 2.988, 95% C.I, P value: 0.012) and not able to regulate the level of blood glucose or increased level of glucose (hyperglycaemic) (AOR: 3.18, 95% C.I, P value: <0.001).

**CONCLUSION:** In patients with diabetes mellitus, the concentration of serum electrolyte was abnormal significantly. In advanced patients of diabetes mellitus, incidence of imbalanced concentrations was more prevalent, with some parameters had a clear strong association with deranged electrolytes in the serum in patients with diabetes mellitus.

**KEYWORDS:** Electrolytes, Risk factors, Diabetes Mellitus, Hyperkalaemia, Hyponatraemia

## INTRODUCTION

Body fluid is an electrolyte- and non-electrolyte-containing aqueous solution having two compartments i.e. extracellular and intracellular. [1-2] Most of the metabolic processes take place mainly inside intracellular fluid (ICF), as a result of which there may be major changes in the strength of the ion with detrimental influences on functions of the body. Extracellular fluid (ECF) acts as a channel efficiently that controls the volume intracellularily and its strength of the ion, requiring most favourable volume maintenance. Whichever change in osmolality extracellularily is followed by equal variation in osmolality intracellularily, followed by a mutual alteration in the cell volume since there is an osmotic balance between extracellular fluid and cells. [1, 3-6]

The electrolytes are compounds which in solution turn into ions and develop ability to carry electricity. [7-8] In several processes, electrolytes work as integral part, including the amount of fluid of the body and regulation of osmosis, rhythmicity and contractility of the myocardial, excitability of the neuromuscular junction and acid-base balance. [1, 8] Important electrolytes in extracellular fluid are sodium ($Na^+$) and chloride ($Cl^-$) ions, though phosphate, potassium and magnesium are essential electrolytes in intracellular fluid. Trans-membrane electrical gradients trigger the cellular $K^+$ diffusion out of the cells and $Na^+$ into the cells. Stimulated through insulin and catecholamine hormones, the $Na^{+-}K^+$ pump overturns the progression of electrolytes to preserve their homeostasis intracellularity and extracellularily.[1, 4, 8-10] Changes in catecholamine and insulin levels influence the amount of serum electrolytes. [5] The mechanisms well thought-out by which solutes and fluid anomalies arise in patients with hyperglycaemia are variations in total quantity of solute extracellularily, osmotic diuresis, thirst-driven intake of water, and influences from related conditions. [11-13]

The most common electrolyte condition is hypo- and hyper-secretion in hospitalised patients. The hyperkalaemia and hypokalaemia occur when the potassium levels in serum is <3.5 mmol/L and >5.1 mmol/L, respectively. The hyponatraemia is called when the serum sodium concentration is <135 mmol/L, and the hypernatraemia is called when the serum sodium concentration is >50 mmol/L. Mechanisms through which enhancement of

the serum may happen are the transfer to the extracellular fluid of electrolytes from the cells or into the cells from extracellular fluid, more intake and decreased renal excretion. [14-19] The diabetes is chronic disease that happens when blood glucose level is elevated as body is not efficiently manufacturing any or adequate insulin or utilize insulin. [20-21] Of the immediate complications of diabetes mellitus caused due to insufficient intake of fluid and total insulin deficiency is the hyperglycaemic hyperosmolar condition, which is characterised by de-hydration, hypotension and hyperosmolarity and can cause disorders of the electrolytes in patients with diabetes mellitus having various periods of hyperglycaemia after several weeks of polyuria. [22-25]

Therefore, the purpose of the research was to evaluate abnormalities of electrolytes in the serum and factors related to abnormalities in diabetic patients.

## PATIENTS AND METHODS

The research was performed from February 2019 to June 2019, at Polyclinic Hospital Islamabad. The research was performed on patients of diabetes mellitus (both type 1 and type 2). A cross-sectional study conducted and a comfort sampling was utilized to provide a number of 155 patients with diabetes mellitus. A questionnaire was used for the collection of clinical history, socio-demographic, and drug use etc. Patients with diabetes mellitus with a record of renal complications or disease as well as patients undergoing care with diuresis while the time of data collection were disqualified from the research. Blood pressure tests were carried out on the basis of recommendations from the World Health Organization (WHO) and patients with diabetes mellitus were deemed hypertensive having elevated diastolic and systolic blood pressure tests. For each study participant, anthropometric assessment was carried out on the basis of WHO guidelines and the body mass index (BMI) was determined using the formula, Body Mass Index = weight over height square and patients with diabetes mellitus were categorised as irregular and average weight (18.5-24.9 $kg/m^2$), obese ≥ 30 $kg/m^2$), overweight (25.0-29.9 $kg/m^2$), underweight (<18.5 $kg/m^2$) according to World Health Organization guideline group.

Every study participant collected five millilitres of over-night fasting blood samples and underwent the requisite standardised methods to isolate the serum from obtained whole blood utilized for determining blood glucose and serum electrolyte. For the determination of serum electrolytes and blood glucose, ion-selective electrode (ISE) device and automated chemistry analyzers were used respectively. To distinguish electrolyte values above and below the standard range, the International Federation of Clinical Chemistry (IFCC) has proposed finishing levels. Reference interval of sodium concentration in the blood is therefore 136-145 mmol/L. Subjects with concentration of sodium ($Na^+$) in serum is <135 mmol/L and >146 mmol/L were classified as hyponatraemic and hypernatraemic, respectively. Same was for concentration of both chloride ($Cl^-$) and potassium ($K^+$) levels in the serum, the reference period is 98-107 mmol/L and 3.5-5.1 mmol/L, respectively. Hypochloraemic and hyperchloraemic individuals were known to be diabetic patients with serum chloride concentrations <98 mmol/L and >107 mmol/L. Similarly, those research participants were classified as hypokalaemic and hyperkalaemic, respectively, with concentration of serum potassium <below 3.5 mmol/L and >5.1 mmol/L.

Before any data review took place, all the questionnaire data was manually analyzed for comprehensiveness and transparency.

After that, all data was entered into SPSS 21.0. In order to determine the intensity of the association between irregular electrolytes in serum and independent variables, Pearson's correlation test was performed. The correlation coefficient values, r= 0.8-1.0, 0.6-0.79, 0.4-0.59, 0.2-0.39 and 0.0-0.19 were considered to be very high, high, moderate, weak and very weak, respectively, depend upon the track of the linear relationship between independent and dependent variables. In order to evaluate and classify independent predictor variables for serum electrolyte abnormality, multivariate and bivariate models of logistic regression were utilized to analyze and classify independent predictor variables for the abnormality of serum electrolyte and certain independent variables with p-values in the model of bivariate logistic were transferred to the model of multivariate logistic. At last, p-value below 0.05 was known to be statistically relevant correlation between independent variables and abnormalities of the electrolytes in serum.

## RESULTS

The age of patients participated ranged from 18 to 75 years (mean age of 48.3 ± 12.9 years). Most of the participants in the sample, males were 52.90% (n= 82/155), though remaining were females. For each subject of the study, three major serum electrolytes were evaluated and in general prevalence rate irregular concentration of serum electrolyte was calculated in 45.80% (n=71/155) patients with diabetes mellitus. Hyponatraemia was the foremost irregular serum electrolyte in patients with diabetes mellitus, accompanied by hypochloraemia (46.47% vs. 19.48%) and only about 14.28% of patients with diabetes mellitus were hyperkalaemic.

Using the Pearson correlation coefficient model, the correlation between the degree of electrolyte concentration in the serum and independent variables was measured. Depend on the evaluation, the amount of potassium ($K^+$), chloride ($Cl^-$) and sodium ($Na^+$) serum electrolyte concentration elevates in period of diabetes mellitus and diastolic hypertension. Age of patients with diabetes mellitus had an effective positive association with concentration of abnormal serum sodium (r=0.712, P=0.003) and weak positive association with concentration of abnormal serum chloride (r=0.081, P=0.311) and a very weak negative correlation with serum potassium abnormal concentration (r=-0.514, P=0.80). The nature of drugs utilized in patients with diabetes mellitus had a clear association to concentration of abnormal potassium in the serum (r=0.911, P=0.001), while the concentration of abnormal sodium in the serum had somewhat poor negative and positive association with the type of drug utilized in patients with diabetes mellitus (r=0.049, P=0.41 vs. r=-0.019, P=0.801). Elevated concentration of potassium in the serum had a clear association with increased BMI (r=-0.008, P=0.141), while concentration of serum chloride and sodium had a somewhat poor association with diabetic patients' elevated BMI (Table 1).

In order to distinguish independent predictor parameters which may contain statistically important correlation with abnormalities of serum electrolyte, regression of multivariate and bivariate logistic was tested after variables whose P values were moved to multivariate logistic regression below 0.25 in regression of binary logistic. Three parameters were classified as containing significant statistically in association with irregular electrolytes in the serum, depends upon regression of multivariate logistic statistical evaluation. Compared to unemployed diabetic patients, working diabetic patients were more likely than unemployed diabetic patients to produce irregular serum electrolytes (AOR: 3.879, 95 % C.I: 1.061–10.49, P-value: 0.044). Likewise, patients with

diabetes mellitus who were treated with both insulin and oral anti-hyperglycaemic agents were more likely than patients with diabetes mellitus treated with insulin alone to establish serum electrolyte abnormality to regulate their blood level of glucose (AOR: 2.988, 95% C.I: 1.301–6.881, P-value: 0.012). In addition, patients with diabetes mellitus with no control in their levels of blood glucose in regular were more likely than patients whose fasting level of blood glucose level was not controlled well or with hyperglycaemics throughout the period of study to experience elevated serum electrolyte levels (AOR: 3.18, 95 % C.I: 2.321-5.812, P-value <0.001) (Table 2).

**Table 1: Association of Serum Electrolytes with Independent Variables (n=155)**

| Predictor Variables | Sodium ($Na^+$) | | Potassium ($K^+$) | | Chloride ($Cl^-$) | |
|---|---|---|---|---|---|---|
| | r-value | p-value | r-value | p-value | r-value | p-value |
| Gender | 0.042 | 0.481 | 0.361 | 0.557 | -0.02 | 0.713 |
| Age | 0.712 | 0.003 | -0.514 | 0.419 | 0.081 | 0.311 |
| Education Level | 0.003 | 0.814 | -0.084 | 0.181 | -0.003 | 0.881 |
| Occupation, | 0.201 | 0.091 | -0.008 | 0.949 | 0.028 | 0.481 |
| Duration of Diabetes Mellitus | 0.094 | 0.202 | 0.0623 | 0.413 | 0.041 | 0.591 |
| Type of Medication | 0.049 | 0.412 | 0.911 | 0.001 | 0.019 | 0.801 |
| Hypertension | -0.091 | 0.381 | -0.001 | 0.861 | 0.581 | 0.510 |
| Body Mass Index (BMI) | 0.218 | 0.018 | -0.008 | 0.141 | 0.041 | 0.719 |
| Systolic Blood Pressure (SBP) | 0.210 | 0.081 | -0.006 | 0.817 | 0.061 | 0.003 |
| Diastolic Blood Pressure (DBP) | 0.071 | 0.418 | 0.007 | 0.002 | 0.841 | 0.318 |
| Alcohol | -0.019 | 0.781 | 0.014 | 0.881 | 0.119 | 0.076 |
| Cigarette Smoking | 0.078 | 0.219 | -0.071 | 0.431 | 0.118 | 0.071 |
| Fasting Blood Glucose (FBG) | -0.312 | <0.001 | 0.118 | 0.071 | -0.319 | 0.002 |

**Table 2: Multivariate & Bivariate Logistic Regression and Related Factors in Patients with Diabetes Mellitus (n=155)**

| Variable | Category | COR | C.I. (95%) | p-value | AOR | C.I. (95%) | p-value |
|---|---|---|---|---|---|---|---|
| Gender | Male<br>Female | 0.841<br>0.983 | 0.441 – 1.210 | 0.288 | 1.661<br>0.981 | 0.951 – 2.991 | 0.089 |
| Age (years) | 18-29 | 1 | - | - | 1 | - | - |
| | 30-39 | 1.077 | 0.371 – 3.101 | 0.901 | 1.498 | 1.374 – 3.018 | 0.778 |
| | 40-49 | 1.441 | 0.588 – 2.719 | 0.419 | 1.440 | 0.606 – 2.771 | 0.411 |
| | 50-59 | 0.891 | 0.414 – 1.810 | 0.804 | 0.901 | 0.441 – 1.771 | 0.776 |
| | ≥ 60 | 0.601 | 0.322 – 1.081 | 0.803 | 0.558 | 0.304 – 1.055 | 0.805 |
| Occupation | Employed<br>Unemployed | 0.401<br>1 | 0.099 – 1.021 | 0.055 | 3.879<br>1 | 1.061 – 10.49 | 0.044 |
| Diabetes Mellitus Type | Type 1<br>Type 2 | 1<br>1.081 | 0.659 – 1.781 | 0.658 | 1<br>0.778 | 0.798 – 4.771 | 0.704 |
| Duration of Diabetes (years) | 5-15<br>≤5 | 1<br>0.804 | 0.388 – 1.771 | 0.541 | 0.778 | 0.378 – 1.887 | 0.776 |
| Type of Medication | Mixed (Insulin + Oral)<br>Insulin | 0.704<br>1 | 0.369 – 1.019 | 0.06 | 2.988<br>1 | 1.301 – 6.881 | 0.012 |
| Hypertension | Yes<br>No | 1.201<br>1 | 0.767 – 1.910 | 0.474 | 1.28<br>1 | 0.756 – 1.887 | 0.501 |
| Body Mass Index (BMI) | Abnormal<br>Normal | 1.189<br>1 | 0.746 – 1.904 | 0.524 | 1.204<br>1 | 0.841 – 1.984 | 0.588 |
| Alcohol | Yes<br>No | 0.691<br>1 | 0.241 – 1.704 | 0.381 | 0.669<br>1 | 0.289 – 1.712 | 0.379 |
| Cigarette Smoking | Yes<br>No | 1.401<br>1 | 0.199 – 9.811 | 0.798 | 1.401<br>1 | 0.189 – 8.998 | 0.803 |
| Fasting Blood Sugar (FBS) | Hyperglycaemic<br>Normoglycaemic | 2.98<br>1 | 0.180 – 0.681 | <0.001 | 3.18<br>1 | 2.321 – 5.812 | <0.001 |

## DISCUSSION

By controlling fluid balance, distribution of oxygen, balance of the acid-base, neurological and cardiac mechanisms, electrolytes participate in maintenance of homeostasis of the body. The electrolyte level in body, however, can be very high or very low, ultimately causing imbalance of electrolyte. In this study, there was a increased prevalence in diabetic patients with one or more electrolyte abnormalities. Of the serum electrolytes measured, most common irregularity in patients with diabetes mellitus was hyponatraemia or low serum sodium concentration. [26-31] Decreased sodium ion concentration in serum of patients with diabetes mellitus may be because of hypovolaemia caused by osmotic diuresis. It is to be implied that glucose has high activity of osmosis, so high concentration of glucose contributes to high osmolality of the serum which allows the water to pass exterior of the cells and leads to dilution of hyponatraemia. Second most common electrolyte deficiency in patients with diabetes mellitus was hypokalaemia. Low serum level of potassium abnormally in patients with diabetes mellitus has also been found in other studies. [32-35] Insulin therapy may be the primary explanation for the elevated level of potassium. Potassium crosses along cell membrane with glucose as insulin is given and the glucose is occupied by cells, reducing the potassium concentration in intracellular fluid as well as in blood. $Na^{+-}K^{+}$ pump, that overturns diffusion of cellular potassium from cells and sodium inside cells triggered by electrical gradients of the transmembrane, is the other possible explanation for hypokalaemia. Two hormones, insulin and catecholamine, stimulate this sodium-potassium pump via β-2-adrenergic receptors, therefore changes in concentration of such hormones may influence convey of $K^{+}$ and its level in the serum. Third irregular electrolyte in serum tested in current study was diabetic hyperchloraemia or elevated serum chloride ion concentration levels in diabetics. From different research, similar results were published. [23, 26]

We also tested the association between the abnormality of serum electrolytes and independent variables. Depend on results, few factors such as smoking, irregular body mass index and age showed a positive association with hyponatraemia, and medication also showed a positive association with hypokalaemia, while smoking was associated with hyperchloraemia. In order to test the relationship between independent and dependent variables, multivariate and bivariate regression of logistic statistics were analysed. Depend on such evaluation, 3 independent variables were established as independent predictor variables for the abnormality of serum electrolyte, specifically diverse medication, elevated fasting level of blood glucose, being used. [31]

## CONCLUSION

The average level of serum concentration of electrolytes was significantly deranged in patients with diabetes mellitus and incidence of abnormalities was highest in advanced-age patients with diabetes mellitus. There was a considerable difference in extent of association between irregular concentration of serum electrolyte and independent variables in patients with diabetes mellitus

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ORIGINAL ARTICLE

# OMEPRAZOLE: A CAUSE OF VITAMIN B12 DEFICIENCY - A HOSPITAL-BASED STUDY

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**ABSTRACT**

This cross sectional prospective research study was done in the Department of Medicine, Indus Medical College, Tando Muhammad Khan. Study duration was from 05 February 2018 to 06 February 2019. It included all patients both female and male. All patients above 15 years of age, of either gender with history of recurrent abdominal pain, dyspepsia or abdominal discomfort, heartburn, GERD, H. Pylori positive patients, patients with gastritis, esophagitis, peptic and duodenal ulcer, bloating and halitosis through outdoor patient department (OPD), were enrolled in the study. The detailed history of all such patients was taken and complete physical and relevant clinical examination was performed. Vitamin B12 deficiency was considered when serum B12 level was <350 pg/ml. The informed consent was taken from every patient or from attendants of patients after full explanation of procedure regarding the study and all such manoeuvres was performed under medical ethics and through the cooperation of whole research team. The study was conducted in compliance with the ethical principles of the Declaration of Helsinki. Venous blood samples were collected in the morning after an overnight fast from 11.00 pm for Vitamin B12 levels. During one year study period, 250 patients on long term use of omeraparzole were studied for vitamin B12 deficiency, of which 143 (57.2%) were males and 107(42.8%) were females. The observed symptoms included recurrent abdominal pain in 38(15.2%), dyspepsia or abdominal discomfort in 40(16%), heartburn in 50(20%), bloating in 13(5%), halitosis in 08(3.2%) and combined / mixed symptoms in 17(11%) patients. Of two hundred fifty patients, 120 (48%) had raised MCV with vitamin B12 deficiency.

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## INTRODUCTION

Proton-pump inhibitors (PPIs) such as omeprazole are one of the most widely prescribed classes of drugs worldwide. PPIs are indicated for treatment of ulcers with or without Helicobacter pylori infection; for treatment of gastroesophageal reflux, Zollinger-Ellison disease, dyspepsia, esophagitis and gastritis; and for prevention of peptic ulcers in patients receiving non-steroidal inflammatory agents (NSAIDs) and in patients with upper gastrointestinal bleeding.[1-2]

Proton pump inhibitors (PPIs) and histamine 2 receptor antagonists ($H_2$RAs) suppress the production of gastric acid and thus may lead to malabsorption of vitamin $B_{12}$. Vitamin $B_{12}$ deficiency was more common among persons with a 2-year or greater supply of PPIs compared with nonusers. [3] Mechanism of action of omeprazole centers on inhibition of the $H^+/K^+$ ATPase enzyme in gastric mucosal parietal cells, which is responsible for hydrogen ion secretion in exchange for potassium ions in the gastric lumen. [4]

$B_{12}$ is a protein-bound vitamin introduced mainly through dairy products and meat that requires the presence of gastric acid and pepsin to be liberated in the stomach. It subsequently binds to R factors forming a non-absorbable complex present in saliva and gastric juice. In the duodenum, the alkaline pancreatic juice that contains proteases brakes up the binding between $B_{12}$ and R factor allowing the cobalamine to bind with the intrinsic factor, a necessary step for it to be absorbed in terminal ileum.[5]

Proton pump inhibitors are among the most commonly prescribed classes of drugs, and their use is increasing, in particular for long-term treatment, often being over-prescribed and used for inappropriate conditions. In recent years, considerable attention has been directed towards a wide range of adverse effects, and even when a potential underlying biological mechanism is plausible, the clinical evidence of the adverse effect is often weak. Several long-term side effects have been investigated ranging from interaction with other drugs, increased risk of infection, reduced intestinal absorption of vitamins and minerals, and more recently kidney damage and dementia.[6] Recent studies have suggested that long-term treatment with PPIs may accelerate the development of atrophic changes of the gastric mucosa, especially in H. pylori-infected individuals.[7] Vitamin $B_{12}$ ($B_{12}$; also known as cobalamin) is a B vitamin that has an important role in cellular metabolism, especially in DNA synthesis, methylation and mitochondrial metabolism. [8] Vitamin $B_{12}$ deficiency is a common cause of megaloblastic anemia, various neuropsychiatric symptoms, and other clinical manifestations. Screening average-risk adults for vitamin $B_{12}$ deficiency is not recommended. Screening may be warranted in patients with one or more risk factors, such as gastric or small intestine resections, inflammatory bowel disease, use of metformin for more than four months, use of proton pump inhibitors or histamine $H_2$ blockers for more than 12 months, vegans or strict vegetarians, and adults older than 75 years.[9]

There is also an association of decreased absorption of vitamin $B_{12}$ in elderly population. Therefore, patients utilizing long-term PPIs and/ or elderly populations will have decreased absorption of vitamin $B_{12}$ from food sources. [10-11] To reduce the likelihood of low levels of $B_{12}$, it is recommended to take $B_{12}$ supplements or ingest $B_{12}$ fortified foods to counteract malabsorption. Side effects associated with low vitamin $B_{12}$ levels include anemia, fatigue, weakness, constipation, loss of appetite and weight loss. [12] To prevent the development of these side effects, it is important to have $B_{12}$ levels monitored when on long term PPI therapy.

The rationale of this study is to explore the association of vitamin $B_{12}$ in the patients taking omeprazole for long duration.There is no data to assist particular care in prescribing omeprazole therapy due to concerns about risk of vitamin $B_{12}$ deficiency with the long-term use of omeprazole. Long-term use of omeprazole does not lead to vitamin $B_{12}$ deficiencies, except possibly in the elderly, or in persons with Zollinger-Ellison Syndrome who are on high doses of omeprazole for prolonged periods of time. There is no convincingly proven data available in our population that omeprazole increase the risk of vitamin $B_{12}$ deficiency so this study has been conducted to determine the frequency of vitamin $B_{12}$ deficiency in patients on long term omeparazole.

It is hypothesized that vitamin $B_{12}$ levels alter in the patients who are taking omeprazole for long duration.

## METHODOLOGY

This cross sectional prospective research study was done in the Department of Medicine, Indus Medical College, Tando Muhammad Khan. Study duration was from 05 February 2018 to 06 February 2019. It included all patients both female and male. All patients above 15 years of age, of either gender with history of recurrent abdominal pain, dyspepsia or abdominal discomfort, heartburn, GERD, H. Pylori positive patients, patients with gastritis, esophagitis, peptic and duodenal ulcer, bloating and halitosis through outdoor patient department (OPD), were enrolled in the study. The detailed history of all such patients was taken and complete physical and relevant clinical examination was performed. Vitamin $B_{12}$ deficiency was considered when serum $B_{12}$ level was <350pg/ml.[17] The informed consent was taken from every patient or from attendants of patients after full explanation of procedure regarding the study and all such maneuvers was performed under medical ethics and through the cooperation of whole research team. The study was conducted in compliance with the ethical principles of the Declaration of Helsinki. Venous blood samples were collected in the morning after an overnight fast from 11.00 pm for Vitamin $B_{12}$ levels. Long-term use of omeparazole (ATC codes A02BC01 through A02BC05) was defined as prescription of >270 Defined Daily Doses (DDD) of PPIs per year for 3 years or more (>810 DDD in 3 years).

The exclusion criteria of the study were; (1) Non-cooperative patients who refused to give consent or participate in the study (2) Patients on metformin therapy, (3) Patients who were on long-term uric acid lowering therapy, and patients who were on potassium intake (4) Anemic patients with the primary disease, such as hepatic disease, haemolytic anemia, cancer, aplastic anaemia, myeloproliferative disease, red cell aplasia, multiple myeloma, leukemia, chronic lung disease, chronic kidney disease and those using immunosuppressive or chemotherapeutic drugs, (5) Pregnant females and alcoholics, (6) Patients with history of resection of stomach or small bowl surgery, (7) Vegetarian population and (8) Patients with malabsorption syndrome and folic acid deficiency. Whereas, to control confounders of the study all patients were also interviewed to exclude known medical problems that couldaffect vitamin $B^{12}$ status and to determine that they had not received cyanocobalamin treatment parenterally.

The collected data was analyzed in SPSS version 18.00. The frequency and percentage of gender and serum $B_{12}$ deficiency in Helicobacter pylori patients was calculated. The mean and standard deviation (SD) was calculated for age. The independent-samples t-test was applied between categorical variables, Chi-square was applied to determine the statistical difference in gender and the p-value <0.05 was considered as statistically

significant. The mentioned statistical tests were applied at 95% confidence interval (CI).

## RESULTS

During one year study period, 250 patients on long term use of omeraparzole were studied for vitamin $B_{12}$ deficiency, of which 143 (57.2%) were males and 107(42.8%) were females. The observed symptoms included recurrent abdominal pain in 38 (15.2%), dyspepsia or abdominal discomfort in 40(16%), heartburn in 50(20%), bloating in 13(5%), halitosis in 08(3.2%) and combined / mixed symptoms in 17(11%) patients. Of two hundred fifty patients, 120 (48%) had raised MCV with vitamin B12 deficiency. The mean age ±SD (ranged) of overall subjects with statistical difference is shown in Table 1 whereas the mean age ± SD of vitamin $B_{12}$ deficient male as well as female subjects was 45.17±11.86 and 46.12±13.01 (P=0.73).

One hundred and thirty (130/52%) patients on omeprazole had normal vitamin $B_{12}$ level and the mean age ± SD of such category's male and female was 45.38±14.16 and 47.21±12.7 (P=0.62). The mean ± SD of serum vitamin $B_{12}$ level in overall subjects (male and female) was 310.717±223.447 pg/ml and 251.342±31.919 pg/ml (P=0.001) respectively.

The mean vitamin $B_{12}$ level on omeprazole subjects with low vitamin $B^{12}$ (male and female) was 136.250±21.423 pg/ml and 142.625±19.969 pg/ml (P=0.22) where as it was 532.459±157.448 pg/ml and 618.170±141.931 pg/ml (P=0.01) on omeprazole subjects (male and female) with normal serum vitamin $B_{12}$ level. The frequency of vitamin $B^{12}$ deficiency observed in on omeprazole patients is shown in Table 2.

In this series, among the patients taking omparazole for 3 years or more, the mean duration of omeprazole use + SD (ranged) was 4.3 ± 0.71 (3 to 8 years) and mean of daily omeprazole dose was 40 mg per day.

**Table 1: Demographic characteristics of the patients (n = 150)**

| | Number | Percentage (%) |
|---|---|---|
| **Age (in years), Mean ± SD** | 44.17 ± 12.9 | |
| **Age Range (in years)** | 15 to 60 | |
| **Age in Groups (in years)** | | |
| 15-30 | 23 | 9.2 |
| 31-40 | 102 | 40.8 |
| 41-50 | 33 | 18 |
| 51-60 | 45 | 13.2 |
| >60 | 47 | 18.8 |
| **Gender** | | |
| Male | 143 | 57.2 |
| Female | 107 | 42.8 |
| **Duration of Omeprazole Use (in years)** | 4.3 ± 0.71 (3-8 years) | |
| **Daily Omerprazole Use** | 40mg | |

**Table 2: Symptoms and Level of Vitamin $B_{12}$ in Patients Using Omeprazole (n=150)**

| | Number | Percentage (%) |
|---|---|---|
| **Symptoms** | | |
| Nausea | 40 | 16 |
| Vomiting | 28 | 11.2 |
| Recurrent abdominal pain | 38 | 15.2 |
| Dyspepsia or abdominal discomfort | 40 | 16 |
| Heartburn | 50 | 20 |
| Bloating | 13 | 5 |
| Halitosis | 08 | 3.2 |
| Combined/Mixed Symptoms | 17 | 11 |
| **Vitamin $B_{12}$ Deficiency** | | |
| Yes | 120 | 48 |
| No | 130 | 52 |
| **Normal Vitamin $B_{12}$ Level** | | |
| Overall, Mean ± SD | 627.361 ± 110.221 | |
| Male | 532.459 ± 157.448 | |
| Female | 618.170 ± 141.931 | |
| **Low Vitamin $B_{12}$ Level** | | |
| Overall, Mean ± SD | 138.362 ± 18.32 | |
| Male | 136.250 ± 21.423 | |
| Female | 142.625 ± 19.969 | |

## DISCUSSION

The advent of proton pump inhibitors (PPIs) has revolutionized the treatment of acid-related disorders, in particular gastroesophageal reflux disease. These drugs have been always considered highly effective and safe, because of the small number of adverse events reported in medical literature. Nevertheless, in the last years the attention of both physicians and patients has been attracted by a mounting number of publications reporting the occurrence of many serious adverse events, particularly with long-term use of PPI therapy.[13,14] In our study, use of omeprazole for 4.3 years was associated with a subsequent new diagnosis of vitamin $B_{12}$ deficiency. The magnitude of the association was stronger in women and younger age groups.

Omeprazole, a substituted benzimidazole, is a specific inhibitor of the enzyme $H^+/K^{+-}$ ATPase, which is found on the secretory surface of the parietal cell. This enzyme, the "proton pump", catalyzes the final step in acid secretion. [15] Gastric acid is required to cleave vitamin $B_{12}$ from ingested dietary proteins for the essential

vitamins to be absorbed, and it is produced by the same cells that produce intrinsic factor, a compound required for vitamin $B_{12}$ absorption. [16] In this study, among the patients taking omeprazole, the mean duration of omeprazole use was 4.3 years with daily omeprazole dose was 40mg per day and 48% found with deficient vitamin $B_{12}$ levels. Similarly, Lewis JR and coworkers revealed that long-term PPI users were more likely to have low vitamin $B_{12}$ levels versus non-users (50% versus 21%, $p = 0.003$). [17] TS Dharamrajan et al concluded that $B_{12}$ status declines during prolonged PPI use in older adults, but not with prolonged H2 blocker use. PPI use was associated with diminished serum $B_{12}$ levels ($P < .00005$). [18] Lam et al. correspondingly demonstrated that long-term PPI use was associated with a doubling in the risk of clinically diagnosed vitamin $B_{12}$ deficiency. [3] Qorraj-Bytyqi H et al concluded that PPIs use for short term therapy did not result in clinically significant iron and/or vitamin $B_{12}$ deficiency; thus, these findings argue routine screening under normal circumstances, although monitoring in elderly and malnourished may be of precious value. [13]

PPIs work by blocking gastric $H^+K^+$-ATPase, which is responsible for pumping $H^+$ ions from within gastric parietal cells into the gastric lumen, where they react with $Cl^-$ ions to form hydrochloric acid. A lack of gastric acid and pepsin decreases the release of vitamin $B_{12}$ from proteins in food and thus reduces its availability for absorption in the ileum. [19] Consequently; omeprazole is PPI and so suppresses the production of gastric acid, may lead to malabsorption of vitamin $B_{12}$. Vitamin $B_{12}$ (cobalamin) deficiency is a common cause of macrocytic anemia and has been implicated in a spectrum of neuropsychiatric disorders. The role of $B_{12}$ deficiency in hyperhomocysteinemia and the promotion of atherosclerosisis is being explored. [20] Proton-pump inhibitors (PPIs) seem to increase the incidence of cardiovascular events in patients with coronary artery disease (CAD), mainly in those using clopidogrel. [21] The spectrum of diseases associated with vitamin $B_{12}$ deficiency is broad and ranges from the absence of symptoms to malabsorption syndrome, pancytopenia and neurological symptoms including paresthesias and signs of myelopathy and/or neuropathy. [22] According to Green R [23] et al, using PPIs for more than three years is related to decreased serum levels of vitamin $B_{12}$. Patients who had been treated with PPIs for more than three years had significantly lower vitamin $B_{12}$ levels than did patients who had been treated for less than three years ($p = 0.022$). No statistically significant differences were found according to the type of PPI ($p = 0.881$ for Esomeprazole, $p = 0.098$ for Omeprazole, and $p = 0.131$ for Lansoprazole), age ($p = 0.937$) or gender ($p = 0.519$).

## CONCLUSION

The use of omeprazole for long period was found to be associated with vitamin $B_{12}$ deficiency. To provide more authentic correlation, study on large population is required Clinicians should adopt the exercise for appropriate vigilance while considering such deficiencies in routine practice, and should prescribe these medications at lowest possible effective dose.

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ORIGINAL ARTICLE

# SIGNIFICANCE OF VACUETTE SRS METHOD FOR THE DETERMINATION OF ERYTHROCYTE SEDIMENTATION RATE (ESR) WITH CONVENTIONAL METHOD AS GOLD STANDARD

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## ABSTRACT

**Objective:** To compare Vacuette (SRS 20/11) ESR values to Conventional/ Standard stergren method with objective of validating the automated and alternative methods.

**Patients and Methods:** This was a cross-sectional analysis, performed at Department of Pathology, Indus Medical College Hospital Tando Muhammad Khan. Manual Westergren process and Vacuette (SRS) methods subjected a total of 120 blood samples to ESR estimations. Results were evaluated on version 21.0 of SPSS. The results were evaluated, and their association was calculated by using the coefficient of Pearson correlation in SPSS.

**Results:** Powerful significant association exists between the Westergren process Vacuette SRS methods with Pearson coefficient 0.96 and highly significant p value <0.001.

**Conclusion:** Vacuette SRS is well associated with the Westergren manual process and is effective and remarkably suitable for employment in clinical laboratories with heavy workload.

**Keywords:** Erythrocyte sedimentation rate, Westergren method, conventional ESR, Vacuette ESR.

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## INTRODUCTION

Erythrocyte Sedimentation Rate (ESR) is one of the most commonly performed investigation in clinical practice. It is increased in plasma (as acute phase proteins) and has cellular characteristics (concentration of RBC, surface change of RBC and aggregation) in different infectious diseases, malignancies, inflammations, and autoimmune diseases. [1-4] They coalesce and induce a disparity in the specific gravity between plasma and red cells, to a greater or lesser degree, and determine the extent to which Rouleaux is formed by the red cells. ESR is a highly responsive measure for chronic as well as silent inflammation which underlies many disease processes. [5-6] Therefore, it is still used as frequent and common investigation even though the accessibility of substitute parameters of inflammation i.e. C-Reactive Protein level and WBC count. Dr R Fahraeus and Dr A Westergren first portrayed the scheme for the ESR in 1921. [7-8] Later, it became popular screening investigation for acute phase proteins as well as chronic illnesses worldwide. To evaluate the ESR there are several different methods, though the traditional Westergren method is at a standstill known as the reference technique. This technique establishes the sedimentation of erythrocytes in a vertically scale tube with specified measurement lengthwise and bore dimension after 1 hour. Although, it is not an automated technique, and also has a hazard of infection, requires somewhat bulky blood volume, and is time consuming with a testing time of 60 minutes (1 hour). [9]

Amplified understanding of biohazards danger to the laboratory personnel has resulted in safer techniques for conducting the ESR, i.e. vacuum operated sample aspiration and automated mixing of sample with sodium citrate (anticoagulant) available in tube. Several techniques were developed for addressing the functional disadvantages of the original Westergren ESR system. These methods calculate the ESR by means of whole blood mixed with citrate or EDTA in dedicated tubes. The erythrocyte sedimentation is measured and re-calculated to Westergren unit (millimetre/hour) afterwards. Benefit of these techniques above Westergren-oriented manual process is that they supply a more readily accessible, fully closed system with automation with performance. This indicates a strong connection with the traditional Westergren process with the Vacuette SRS system. [10-11] After 30 minutes, the Vacuette (SRS 20/11) technique evaluates the results. These methods show strong association with Westergren's traditional reference system. This study was conceived to compare Vacuette SRS 20/11's ESR values with traditional Westergren process, with the goal of validating Vacuette SRS method.

## PATIENTS AND METHODS

The Department of Pathology, Indus Medical College Hospital, Tando Muhammad Khan, conducted a cross-sectional analysis from February 2019 to May 2019. Westergren subjected a total of 120 samples to ESR estimation, and 20/11 methods of Vacuette SRS. Blood was treated with citrate solution for both of the two processes. The SRS 20/11 vacuette works by measuring 30-minute time. The Westergren ESR was performed using sodium citrate as an anticoagulant using the International Council for Standardisation for Hematology (ICSH) selected procedure. Results for the study were entered on SPSS version 21.0. Based on the ESR values obtained by Westergren process, we divided our patients into three groups: Group 1: ESR 0-20 (mm/h); Group 2: ESR 21-50 (mm/h); Group 3: ESR 51-100 (mm/h). Means of results extracted from automated and manual techniques were

evaluated in all samples. The p values were determined in three groups; p Value of <0.05 was considered significant statistically. For both methods variation coefficient has been determined. The Pearson correlation for the Vacuette SRS System was determined.

## RESULTS

The mean and standard deviation values mean difference and CV determined for the Westergren and Vacuette SRS methods are shown in Table 1 in total samples and in three classes. In the Vacuette SRS system, the disparity was important with the group 2 (ESR 21-50 mm/hr) Vacuette SRS process. The Pearson correlation between distinct methods is expressed in Table 2. There is a clear positive association between the Westergren method and the Vacuette SRS method with a Pearson coefficient of 0.96 and a highly significant p value of <0.001, as shown in the table 2.

**Table 1: Results of Various Groups (n=120)**

| Method | Mean ± SD (mm/time) | Difference of Means | P value | CV (%) |
|---|---|---|---|---|
| **All cases (n=120)** | | | | |
| Westergren | 24.93 ±20.81 | 3.12 | <0.03 | 93.32 |
| Vacuette SRS | 21.81 ± 19.22 | | | 96.11 |
| **Group 1 (n=75)** | | | | |
| Westergren | 9.67 ±4.23 | 0.75 | 0.12 | 60.03 |
| Vacuette SRS | 8.92 ±4.98 | | | 59.16 |
| **Group 2 (n=34)** | | | | |
| Westergren | 34.55 ± 8.32 | 7.43 | 0.003 | 23.12 |
| Vacuette SRS | 27.12 ± 7.56 | | | 33.05 |
| **Group 3 (n=11)** | | | | |
| Westergren | 76.32 ±12.65 | 3.2 | 0.11 | 16.41 |
| Vacuette SRS | 73.12 ±13.43 | | | 24.55 |

**Table 2: Correlation between Both Methods (n=120)**

| Method | Correlation | P value |
|---|---|---|
| Westergren method | r = 0.96 | <0.001 |
| Vacuette SRS Method | | |

## DISCUSSION

Erythrocyte sedimentation rate (ESR) is a reasonably easy and economical investigation performed to evaluate patients with acute and chronic inflammatory processes. [12-13] ESR has been revealed to link with an adverse prognosis in malignant conditions and cardiac malfunction as a helpful support in the identification of a variety of clinical situations. [14-17] In consideration of the need for standardisation of the ESR calculation, ICSH introduced a code of behaviour for evaluating substitute techniques alongside the reference technique: the new methods must be evaluated on spectrum of 2–120 mm ESR level. 95% of the variations in this contrast must be ≤5 mm, with greater variations correlated with elevated ESR levels. The statistical modes suggested for the ESR assessments are correlation coefficient, Passing-Bablock regression and statistical approach BlandAltman. [18] We conducted the analysis to come across for similarities between Vacuette SRS and Westergren process. With very considerable p-value of <0.001, we observed clear positive association of Vacuette SRS method with Westergren rule. We have measured Variance Coefficient for various methods. The excellent association between Automated and Westergren was also verified by other researches. [19-20]

Several other automated techniques are used currently. Horsti J conducted a research and confirmed that Starr-sed had benefits because it provides reserves in consumables, protection and smooth workflow. The Starr-sed has numerous outstanding practical characteristics, and analysis showed reasonably strong association between the two techniques (R2 = 0.72) and revealed that the Westergren approach is improved suited to StaRRsed. They stated that the variations found in their analysis between StaRRsed and the classic Westergren approaches were appropriate and significant clinically. [21] The Fiorucci also evaluated the Westergren method to the Test 1 framework, but the findings revealed a lower level of conformity between the two techniques. Although results acquired with the Test 1 system are surrounded by rational limits, they indicated that the Ves-Matic instrument revealed a stronger association with Westergren process, thereby reducing the probability of false negative and positive results. [22] Although, Curvers et al stated less association with the Westergren technique of Ves-Matic, while a strong association with the traditional method of the SEDI system and StaRRsed methods. [23]

Our analysis showed the subordinate levels and mean difference between three techniques in three groups, especially at higher ESR values, by the Vacuette SRS method compared to the Westergren method. It was proposed by Subramanian et al to add a correction factor to the spectrum of the ESR levels with such divergences. [24] 14 samples in group 3 and 29 in group 2 were reported only. Because these 2 classes are generally significant as they include the diseases with pathological basis suggesting ESR levels. Our suggestion is to perform more studies in each group with a minimum of 50 samples, and the third group should also include more than 100 ESR samples according to the Westergren process. However, both of the methods show strong association with the Westergren method with current available data.

## CONCLUSION

In conclusion, the Vacuette SRS method shows a strong correlation (as shown by the Pearson correlation coefficient) with the Westergren process. These findings show that for a high workload clinical laboratory, Vacuette SRS method is efficient and suitable systems.

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ORIGINAL ARTICLE

# CO-EXISTENCE OF MALARIA WITH THROMBOCYTOPENIA

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## ABSTRACT

**BACKGROUND:** Malaria, especially in developing countries, is an important health problem. It is significant cause of mortality and morbidity, particularly in tropics. In this specific disorder, there are many haematological changes, including thrombocytopenia, anaemia, atypical lymphocytosis as well as rare intravascular coagulation.

**OBJECTIVE:** Purpose of this study is to identify and evaluate the extent of different haematological variations, particularly additional findings of thrombocytopenia in a specific form of malaria.

**METHODS AND MATERIALS:** This was an observational study carried out at Department of Pathology, Indus Medical College Hospital Tando Muhammad Khan, on patients attending the clinically suspected malaria outpatient and inpatient departments. Thin and thick peripheral smear slides for malarial parasites were developed and were stained with Leishman's stain, followed by antigen tests were also incorporated where possible. Total blood counts were used in other studies.

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**RESULTS:** 238 (28%) patients showed malaria parasites on peripheral blood film among the 850 patients with suspected malaria included. Male superiority of 2.01:1 was observed as compared to females. A total of 196 out of 238 patients had thrombocytopenia. Higher levels of thrombocytopenia were observed in P. Vivax (62.26%) is accompanied with Mixed-type Infection (21.42%) and Plasmodium falciparum (15.81%).

**CONCLUSION:**
The anaemia specifically with decreased count of platelets (thrombocytopenia) was observed specifically in Plasmodium vivax, accompanied with Plasmodium falciparum. Given that thrombocytopenia is linked with patients of malaria, as shown in current research, in patients having low platelet count and fever, the care of doctors should preserve malaria as one of the differential diagnoses.

**KEYWORDS:** Malaria, Thrombocytopneia, Plasmodium falciparum, Plasmodium vivax.

## INTRODUCTION

In particular, in Asian and African developing countries, malaria is important health concern. It is significant reason of mortality and morbidity, particularly in the tropical regions. Despite advances in technical expertise, this appears as major issue among majority of inhabited countries, placing financial strain on countries troubled. [(1)] Approximately, more than 40 percent of population of the world is estimated to live in areas in which malaria is endemic and 300-500 million patient cases and 1.5-2.7 million fatalities are expected to happen yearly. [(2)] In extreme malarial types, the fatality ratio is augmented by 20% (parasitaemia > 5%) [(3)]. As the haematological system is key target for malaria, a range of malaria-related complications are seen, and such problems play an important role in causing severe complications. They play an important role in contributing to severe complications. Anaemia, atypical lymphocytes, thrombocytopenia and, to a slighter extent, disseminated intravascular coagulation (DIC) are all anomalies. [(4)] Leucopaenia, leukocytosis, eosinophilia and monocytosis are other observed findings. In patients suffering from malaria, thrombocytopenia comprises 70%, extreme anaemia 25%, cell count in the blood may be high, low or normal i.e. elevated count of the blood cells constitutes <5%, which is taken as a weak factors in prognosis. [(5)] Thrombocytopenia is most frequent among the reported haematological complications and is observed in Plasmodium falciparum as well as vivax. Whilst thrombocytopenia is found in peripheral film, that in patients with fever lacks the usual amount, it is an indicator that patient is infected from malaria. [(4)] As the accurate mechanism of thrombocytopenia is not completely known, it is believed that immune-mediated breakdown of the cells, spleen sequestration, and dyserythropoeitic processes in bone marrow with reduced development of platelets take part in the mechanism. [(5)] Purpose of the research was to identify and evaluate the extent of different haematological variations, particularly in case of thrombocytopenia in a specific form of malaria.

## PATIENTS AND METHODS

It was a cross-sectional observational research was carried out from December 2018 to June 2019 at Department of Pathology, Indus Medical College Hospital Tando Muhammad Khan. Research included clinically suspected malaria cases in patients presenting with fever. Patients with bleeding problems, drugs, thrombocytopenia, chronic liver disease attributes were excluded. Using the automated Mindray BC5000 Haematology Analyzer unit, blood parameters were computed. Two slides were used to make thick and thin blood films, stained with Leishman's dye, and the malarial parasites were recognized and confirmed for

diagnosis of malaria. Peripheral smear slides that were malaria positive were reviewed for further validation from pathologists, defining the particular type of organisms. Among slides studied, including ring form, schizonts form and trophozoites form, all stages of haemoparasite were recognized. In Plasmodium vivax species and gametocyte form in Plasmodium falciparum species, ring forms and trophozoites were primarily observed. Final re-evaluation has been carried out for platelet counts. SPSS 21.0 was used for statistical analysis.

## RESULTS

196 (23.05%) patients showed malaria parasites on peripheral blood film among the 850 patients with suspected malaria included. The overall males included were 131 (66.83%), while females were 65 (33.16%). The predominance of male was observed (ratio of 2.01:1). Groups for age were classified in 7 groups and average age was 18.2 years, ranging from 1-70 years. The highest number of cases reported was between 21 and 30 years of age and less than 10 years in the least common age group (Table 1).

Of total of 196 individuals, 122 (62.26%) had Plasmodiaum vivax infection and 42 patients (21.42%) had a mixed-type infection with Plasmodium vivax and Plasmodium falciparum (Table 2). Of the total, 196 were having thrombocytopenia (82.35%). In both types of malarial species, thrombocytopenia has been identified (Table 3). Individuals with Plasmodium vivax infection and mixed-type infection had a substantial reduction in grade III platelet counts, consisting of 65.55% of P. vivax cases and 31.11% of mixed infection cases 238 cases showed smear-related malaria infection and 196 patients had thrombocytopenia. Sensitivity and precision were 89.9% and 87.5%, respectively.

**Table 1: Allocation of Patients by Age Groups (n=196)**

| Age Group (in Years) | No. of Patients | Percentage (%) |
|---|---|---|
| <10 | 6 | 3.06 |
| 11-20 | 26 | 13.26 |
| 21-30 | 74 | 37.75 |
| 31-40 | 59 | 30.10 |
| 41-50 | 21 | 10.71 |
| 51-60 | 4 | 2.04 |
| >60 | 5 | 2.55 |

**Table 2: Distribution of Malaria Species (n=196)**

| Species of Malaria | Frequency (No.) | Percentage (%) |
|---|---|---|
| Plasmodium Vivax specie | 122 | 62.26 |
| Plasmodium Falciparum specie | 31 | 15.81 |
| Mixed Plasmodium Infection | 42 | 21.42 |

**Table 3: Grading of Thrombocytopenia According to Plasmodium Species (n=196)**

| Platelets (in cumm) | Grading of Thrombocytopenia | Plasmodium Vivax specie | Plasmodium Falciparum specie | Mixed Plasmodium Infection |
|---|---|---|---|---|
| 75,000 – 150,000 | Grade I | 12 | 2 | 3 |
| 50,000 – 75,000 | Grade II | 31 | 4 | 8 |
| 5,000 – 50,000 | Grade III | 57 | 3 | 28 |
| <25,000 | Grade IV | 21 | 22 | 5 |

## DISCUSSION

In most parts of the world, malaria seems to be a big health burden, primarily by Plasmodium vivax and falciparum that is endemic among several countries in Africa, Asia, and Nepal. Characteristic leading to diagnosis may be malaria that causes many haematological anomalies, such as thrombocytopenia and anaemia. [6] In comparison to the previous studies that recorded a average age of 38 years, out-of-patient numbers in current study were among ages of 21-30 years of age. Age and gender information is limited, but few researches have revealed an increased involvement of male gender in comparison to the female gender, and the current research has revealed similar results. [1, 7] Dhungat et al revealed a male-female fraction of 68% and 32% respectively that is about in near alignment with current research (Males-66.33% and Females-33.16%). One of main variables for superior hazard in males is outdoor activity and less mosquito bite protection. [8]

P. vivax has been confirmed to be the primary malaria parasite for the disease process in the subcontinents. [9] Various studies have reported Plasmodium vivax to be prevailing specie, though the percentage of positive cases varies (56.5%; 69% and 51.6%). In the current analysis, similar results were obtained. In the north-eastern areas of Asia, though, an increased prevalence of Plasmodium falciparum has been identified. [8-13]

Decrease in platelet count (thrombocytopenia) is a typical characteristic of acute malaria, as found in our research. It can occur irrespective of severity in Plasmodium vivax as well as Plasmodium falciparum. [4] Conflicting amount of decrease in platelet count in circulating is consistently observed in various malaria species. A drop in the usual amount of platelets relies on peripheral film followed by fever that in the case of malarial infection is a diagnostic clue. [14] It is used in patients suffering from fever of unknown origin as an indication of malaria. [15] Patients with malaria who acquire thrombocytopenia rarely bleed, regardless of the degree of reduction in count of platelet [16], however, on the divergent; no bleeding deaths were observed in the current study.

Several observational studies have been carried out and have also established the correlation of thrombocytopenia with malaria; however the accurate reason of thrombocytopenia is still hard to identify. Reason and correlation of thrombocytopenia with disturbances in coagulation profile, splenomegaly, oxidative stress, alteration in the bone marrow, and antibody-mediated destruction of platelet have been identified

as several causes. [17-18] As also observed in our research, increased tolerance to decreased count of platelets is seen in malaria. No bleeding trends and losses were found in the patients, despite the much decreased count in platelets (up to 20,000/cumm). In many trials, thrombocytopenia has not been linked with any fatality or complications by bleeding, including the present one. [19] The tolerance of low platelet counts in malaria has been clarified by increased platelet activation and aggregation. [7] Increased haemostatic reactions due to hyperactive platelets, despite a substantial reduction in the count of platelets, have been clarified as explanation for uncommon bleeding manifestations in acute malaria. [19] Several studies were performed on thrombocytopenia scoring that was actually dependant on the amount of reduced count of platelets comparable to current research. [20-22] Khan et al performed research in which highest frequency of individuals in grade I was 21%, whilst the major category in the current research was among group III, consisting of 37.75%. [20]

## CONCLUSION

Anaemia specifically with decreased platelet count (thrombocytopenia) was observed specifically in Plasmodium vivax, followed by Plasmodium falciparum infection. As thrombocytopenia is correlated with malaria, as shown in current research, among patients with reduced platelets and fever, the care of doctors should preserve malarial infection among differential diagnosis.

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ORIGINAL ARTICLE

# HYPOMAGNESAEMIA IN DIABETES MELLITUS TYPE 2

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## ABSTRACT

**Introduction**: Patients of diabetes mellitus type 2 have insufficiency of many important elements including serum magnaesium. Hypomagnesaemia is related with complications of diabetes mellitus and amplified the duration of disease.

**Objective:** Main objective of the study is to evaluate the level of serum magnaesium levels and its association with glycated haemoglobin (HbA1c) in patients of diabetes mellitus type 2.

**Methodology:**

The study was prospective, carried out at Department of Pathology and Department of Medicine, Indus Medical College Hospital Tando Muhammad Khan between the duration of January 2018 to June 2018. 265 individuals were selected for this study and were classified as control group (n=135) and diabetic group (n=130). Samples of all participants were collected in EDTA-containing tube and Gel tube for the evaluation of HbA1c and serum magnaesium respectively. The variables were evaluated and analyzed in SPSS 21.0. The p – value of <0.05 was taken as significant statistically.

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**Results:** Males in the study were 154 (58.1%) and females were 111 (41.88%). Mean age of the included participants in control and diabetic group was 48.3 ± 3.77 years and 49.10 ± 4.98 years respectively. Mean glycated haemoglobin in control group was 5.89 ± 1.02 %, and in diabetic group was 8.71 ± 1.86 %, with statistically significant association (p <0.001). The serum magnaesium level in control and diabetic group was 1.89 ± 0.25 mg/dL and 0.58 ± 0.13 mg/dL, with statistically significant correlation (p <0.001).

**CONCLUSION:**

Hypomagnesaemia was associated with increased level of glycated haemoglobin showing its strong correlation with glycaemic control.

**KEYWORDS:** Magnaesium, hypomagnesaemia, diabetes mellitus, insulin, glycaemic control, glycated haemoglobin.

## INTRODUCTION

Magnaesium (Mg) is one the main electrolytes and is the most plentiful divalent intracellular cation in cells, and comes next to potassium as cellular ion. [1] Total magnaesium is present in three distinctive forms namely; protein – bound, complexes and free cation. The most abundant form of magnaesium is intracellular type which is cofactor of various enzymatic processes. Magnaesium – ATP complex plays very essential role in metabolism of the body, contraction of muscles, transfer of methyl groups and others. Magnaesium is also required for the action of insulin signal, proliferation of cells and is important for the membranes of calcium ($Ca^{2+}$), sodium ($Na^{+}$) and potassium ($K^{+}$) ions. [2] It also plays important part in homeostasis of glucose and sensitivity of insulin in type 2 diabetes mellitus. Evidence shows low level of magnaesium in diabetic patients; and is especially related to neurological complications of the diabetes mellitus. [3]

In type 1 diabetes mellitus patients with proteinuria or microalbuminuria, significant decrease in ionized magnaesium has been found. [4] Low intake and increased loss of magnaesium usually favours depletion of magnaesium ($Mg^{+2}$) in patients with diabetes mellitus; though absorption as well as preservation of magnaesium ($Mg^{+2}$) is mostly not affected. [5] Therefore, low level of magnaesium may cause worsening of diabetes mellitus and can even induce more hypomagnesaemia. Evidence suggested low levels of magnaesium in diabetic patient as compared to non – diabetic controls and may also be caused because of resistance to insulin and systemic inflammation. [6] Due to osmotic diuresis, magnaesium is lost frequently. Hypomagnesaemia is also related inversely to glycaemic control. Past evidences showed low risk of complications in diabetic patients by giving adequate magnaesium intake. [7]

Here we conducted a study to compare serum magnaesium ($Mg^{+2}$) concentration in patients with diabetes mellitus type 2 and controls (non-diabetics).

## PATIENTS AND METHODS

The study is a prospective cross – sectional, performed at Department of Pathology and Department of Medicine, Indus Medical College Hospital Tando Muhammad Khan. A total of 130 diabetes mellitus type 2 patients, and 135 controls were selected for the study. Study was conducted between the periods of 6 months (January 2018 to June 2018). All patients with diabetes mellitus type 2 with no co-morbid conditions were integrated in the study. Patients aged between 30 years and 70 years were included. 135 normal controls were also included. Patients with renal, cardiac, neurological or other complications were excluded from the study.

Overnight fasting sample of 5mL venous whole blood was extracted by aseptic measures and standard procedure. The 2.5 mL blood was shifted to collection tube containing lithium

heparin. Serum was separated without any delay by centrifugation. Samples were analyzed for serum magnaesium. Remaining 2.5 mL was shifted to collection tube containing EDTA and was used for analysis of HbA1c. Magnaesium and HbA1c were measured using COBAS C111 Chemistry Analyzer Roche. Data was analyzed using SPSS 21.0. Data was presented as mean, standard deviation etc. Parameters were evaluated and compared using independent t-test. The p – value of <0.05 was considered as significant statistically.

## RESULTS

Among 265 participants, 154 (58.11%) were male while 111 (41.88) were females. All participants were divided into two groups: control and diabetic groups. Control group consisted of 135 normal individuals. Out of 135 control individuals, 79 (58.51%) were male while 56 (41.48%) were female (Figure 1). Diabetic group consisted of 130 diabetic individuals. Out of 130 diabetic group, 75 (57.69%) were male and 55 (42.30%) were females. Mean age in control and diabetic groups was 48.3 ± 3.77 years and 49.10 ± 4.98 years respectively. P – value was not statistically significant. In control group, mean glycated haemoglobin was 5.89 ± 1.02 %, while in diabetic group it was 8.71 ± 1.86 %. P – value was <0.001. In control group, mean serum magnaesium level was 1.89 ± 0.25 mg/dL, while in diabetic group it was 0.58 ± 0.13 mg/dL. P – value was <0.001.

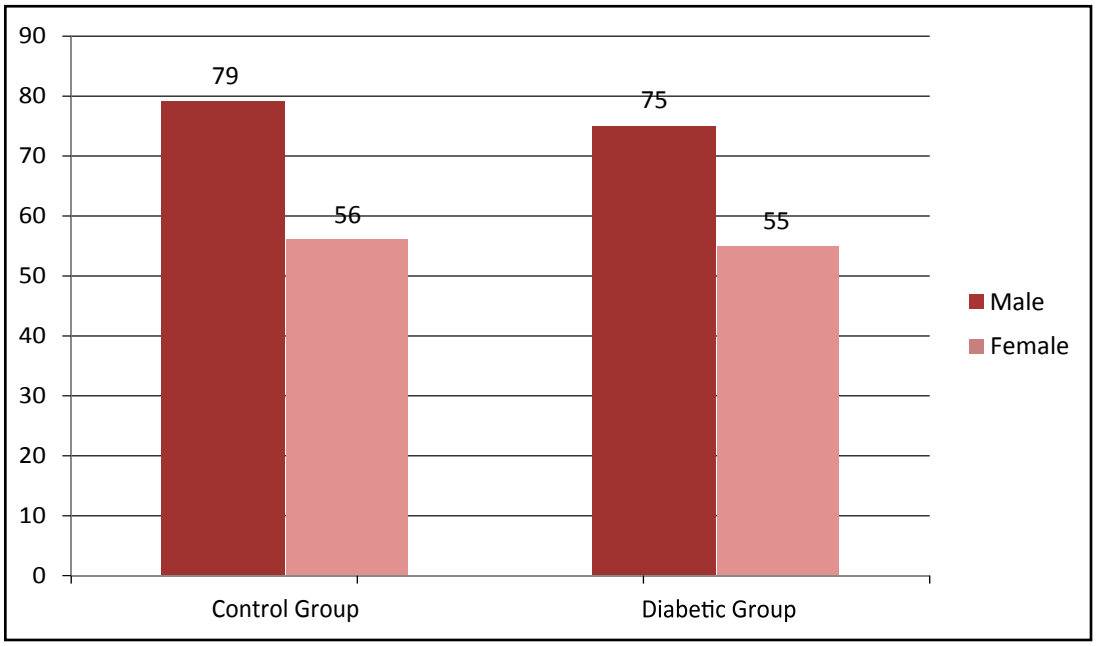


**Figure 1: Gender Distribution (n=265)**

**Table 1: Comparison of Serum Magnaesium Levels in Control and Diabetes Groups (n=265)**

| | Control Group | Diabetic Group | P – value |
|---|---|---|---|
| Age (years) mean | 48.41 ± 3.77 | 49.10 ±4.98 | 0.794 |
| HbA1c (%) mean | 5.89 ± 1.02 | 8.71 ± 1.86 | <0.001 |
| Serum magnaesium (mg/dL) mean | 1.89 ± 0.25 | 0.58 ± 0.13 | <0.001 (Table 1) |

## DISCUSSION

Magnaesium is one of the most important elements for the body. It acts as cofactor in more than 300 enzymatic processes, especially involves phosphate group transfer. It has important part in maintenance of cardiac function and excitability of neuromuscular junctions. [8] This study showed significant difference of serum magnaesium levels in context of HbA1c levels in diabetic patients in comparison of control group (p – value <0.001). Murthy et al showed in his study that low level of magnaesium was significantly related to diabetic nephropathy as well as retinopathy. Serum Magnaesium level was decreased in diabetic patients as compared to control group. [9] Manikandan et al showed in his study that level of serum magnaesium was low in diabetic patients and were significantly associated with microvascular and macrovascular complications. [10] Omero et al proved in his study that supplementation of oral magnaesium replenished level of serum magnaesium, sensitivity to insulin and metabolic control in diabetic patients who had initially reduced level of magnaesium. [11] El-said demonstrated that hypomagnesaemia was closely associated with diabetic mellitus and glycaemic control. [12] Noor et al showed that hypomagnesaemia was present in diabetic patients and was associated with increased duration of diabetes mellitus. [7] Nair et al showed correlation of serum magnaesium with various factors. He demonstrated that low level of magnaesium was associated with diabetes patients with hypertension and other related complications i.e. retinopathy, cellulitis, neuropathy etc. Level of glycated haemoglobin was significantly high in patients with low magnaesium levels. [13]

Mechanism of deficiency of magnaesium in the progression of complications of diabetes mellitus has not been understood completely. Level of serum magnaesium is associated with resistance of insulin and function of β – cells in patients with diabetes mellitus. Likewise, deficiency of magnaesium is linked to decreased function of β – cells and increased resistance to insulin that lead to increased level of plasma glucose. [14] Depletion of serum magnaesium has negative correlation with homeostatic of glucose and insulin sensitivity. Hypomagnesaemia may alter the transport of cellular glucose by varying $Na^{+-}K^{+-}$ATP gradients, decrease secretion of pancreatic insulin, defective signalling of post – receptor insulin and decrease the interaction of insulin – to – insulin receptors. Hypomagnesaemia is also shown to destroy the activity of tyrosine kinase and receptors capable of signalling. [15] Present study demonstrated the same findings of decreased level of serum magnaesium in diabetic patients suggestive of its strong correlation with glycaemic control.

## CONCLUSION

Hypomagnesaemia was found to be associated with increased glycated haemoglobin in diabetic patients in our study. Considering serum magnaesium estimation as routine diagnostic and monitoring panel of diabetic patients may determine the early hypomagnesaemia and diabetic complications. Early intervention by supplementary magnaesium diet or therapy may reverse or control the effects produced by low level of magnaesium.

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REVIEW ARTICLE

# RECENT ADVANCES IN DIAGNOSIS OF THALASSEMIA

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**ABSTRACT**

Mutations and/or deletions in the α-globin or β-globin genes cause hereditary hemoglobin disorders. Thalassemia is caused by haemoglobin structural abnormalities due to quantitative abnormalities, and hemoglobinopathies. With a rapid influx of people from endemic areas and marriages in blood relations, the incidence of thalassemia and hemoglobinopathy is growing. Therefore the disease knowledge is required. The α-thalassemias are induced by α-globin gene deletions, whereas β-thalassemias are associated with reduced β-globin synthesis due to mutations in the β-globin gene. Hemoglobinopathies include structural changes of hemoglobin in the α- or β-globin chains due to altered amino acid sequences. The next step is to detect hemoglobin abnormality using electrophoresis techniques, including high-performance liquid chromatography and mass spectrometry, if the patient is suspected of thalassemia / hemoglobinopathy from irregular complete blood count findings and/or family history. A more accurate molecular diagnosis of thalassemia / hemoglobinopathy is enabled by the advancement of innovative molecular genetic technologies, such as massively parallel sequencing. In addition, genetic testing for prenatal diagnosis allows the prevention of birth and pregnancy complications from thalassemia. The goal was to review the range and classification of diseases of thalassemia / hemoglobinopathy and diagnostic methods, including screening tests, molecular genetic tests, and prenatal diagnosis.

**Keywords:** Hemoglobinopathies, thalassemia, diagnosis, genetic testing, advances.

## INTRODUCTION:

Biconcave disk-shaped cells without nuclei are red blood cells (RBCs) and are the most common cells present in the blood. RBCs provide peripheral tissues with oxygen and thus play a vital role in sustaining the life of organisms. Hemoglobin molecules in the cytoplasm of RBCs, which are highly advanced devices that bind, hold, and release oxygen, are used to transport oxygen. A hemoglobin molecule consists of 4 polypeptide globin chains (2-α and 2-β) each containing within a heme molecule that binds to oxygen. The hemoglobin molecules and RBCs are essential for the quantitative balance between the

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globin chains and their structural stability. Hemolytic anaemia occurs when the genes encoding the globin chains (HBA for α-globins and HBB for β-globins) have mutations (pathogenic variants) contributing to changes in the volume or structure of globins. Thalassemia is the most common type of human hemoglobinopathy and is caused by pathogenic variants leading to faulty protein synthesis, resulting in quantitative imbalance in globin chains. [1-2] Other pathogenic variants which cause structural abnormalities in globin chains cause hemoglobinopathies like sickle cell disease. [3]

Diagnosis of thalassemia / hemoglobinopathy starts with suspicion of the disorder in anaemic patients based on the findings of phenotype, family history and related laboratory test screening. Molecular genetic confirmation confirms the diagnosis by identifying pathogenic variants. In the past, the genetic variability of the disorder and the mutations which were overlooked by traditional sequence analyses complicated the molecular genetic diagnosis. However, the recent advent of novel molecular genetic technologies such as dosage mutation tests to detect significant deletion / duplication mutations and multiple massively parallel sequencing gene panel tests has allowed a more reliable molecular diagnosis of hereditary hemolytic anaemia and a deeper understanding of the disease's genetic / genomic mechanisms. [4-5] The spectrum and classification of thalassemia / hemoglobinopathy diseases and diagnostic methods, including screening tests, molecular genetic tests and prenatal diagnosis, were studied in this review.

## CLASSIFICATION

Hemoglobinopathies are defects of hereditary hemoglobin caused by α-globulin or β-globulin gene mutations and/or deletions. These are classified into 2 major categories: structural hemoglobin variants and thalassemias. Thalassemias, classified as α- or β-thalassemia depending on the involved α- or β-globin chain, are caused by synthesis defects of hemoglobin chains. Structural variants of hemoglobin including sickle cell disease, hemoglobin C (HbC) disease, and hemoglobin E (HbE) disease are caused by gene defects that modify the structure of the hemoglobin. Furthermore, the symptoms of both thalassemia and structural types are combined in several mixed ways (Table 1). [6-7]

The α-thalassemia is almost the product of partial ($\alpha^{+}$) deletions or complete ($\alpha^{0}$) deletions of the α-globin gene. People with more deletions of the gene show more serious clinical symptoms. Fatal hemoglobin Bart's foetal hydrops, associated with homozygous $\alpha^{0}$ -thalassemia, are typically characterised by extreme hemolytic anaemia, hydrops and ascites in or shortly after birth, which may lead to foetal death. [8-9]

The β-thalassemias caused by β-globin gene mutations are associated with incomplete ($\beta^{+-}$ $\beta^{++}$) or absent ($\beta^{0}$) synthesis of β-globin. The severity of symptoms is related to the degree of absent β-globin chain development. [10]

Due to the altered amino acid sequence in the α- or β-globin chains, irregular hemoglobins have structural defects, unlike thalassemias. Hemoglobin S (HbS), HbC, and HbE are among the common hemoglobin abnormalities. Of all hemoglobinopathies, HbS is the most dangerous. The sickle cells might cause vascular obliterations and infarctions in the main organs. [11] The path of HbC disease is close to progression of sickle cell disease but less fatal. The HbE condition, however, is like β-thalassemia. Other abnormal hemoglobins with structural detection are available.

**Table 1: Classification of Hemoglobinopathies**

| Type | | Diagnosis | Gene Type |
|---|---|---|---|
| Thalassemia | α-thalassemias | Heterozygous $\alpha^{+}$-thalassemia | -α/αα |
| | | Homozygous $\alpha^{+}$-thalassemia | -α/-α |
| | | Heterozygous $\alpha^{0}$-thalassemia | --/αα |
| | | Mixed heterozygosity, $\alpha^{2}/\alpha^{2}$ - thalassemia | --/-α |
| | | Homozygous $\alpha^{0}$- thalassemia | --/-- |
| | β-thalassemias | Heterozygous β-thalassemia | $\beta^{++}/\beta$, $\beta^{+}/\beta$, $\beta^{0}/\beta$ |
| | | Mild homozygous or compound heterozygous β – thalassemia | $\beta^{+}/\beta^{+}$, $\beta^{+}/\beta^{++}$, $\beta^{+}/\beta^{0}$, $\beta^{0}/\beta^{0}$ |
| | | Homozygous β – thalassemia | $\beta^{+}/\beta^{+}$, $\beta^{0}/\beta^{0}$ |
| | | Compound heterozygous β – thalassemia | $\beta^{+}/\beta^{0}$ |
| Structural variants | HbS | HbS heterozygosity | HbAS |
| | | Sickle Cell Disease | HbSS |
| | HbC | HbC heterozygosity | HbAC |
| | | HbC disease | HbCC |
| | HbE | HbE heterozygosity | HbAE |
| | | HbE disease | HbEE |
| Mixed variants | β-thalassemia + HbS or HbE | Sickle cell $\beta^{+}$-thalassemia | HbS $\beta^{+}$-thalassemia |
| | | Sickle cell $\beta^{0}$-thalassemia | HbS $\beta^{0}$-thalassemia |
| | | HbE $\beta^{+}$-thalassemia | HbE $\beta^{+}$-thalassemia |
| | | HbE $\beta^{0}$-thalassemia | HbE $\beta^{0}$-thalassemia |
| | HbS + HbC | HbSC | HbSC disease |

## COMPLETE BLOOD COUNT

Mean corpuscular volume (MCV) of less than 80 fL and/or mean corpuscular haemoglobin (MCH) of less than 27 pg can typically be used for thalassemia screening as cut-off thresholds for a positive screening result. [12] These cut-off thresholds are derived from 2 standard deviations from the general population of the normal distribution of MCV and MCH. The benefit of MCV and MCH thalassemia screening is the achievement of quick, cost-effective, reproducible and precise results from automated hematology analyzers. However, microcytic anemias such as iron deficiency anemia (IDA) can also cause low MCV; variation in MCV from different automated blood cell counters has also been documented in comparison to MCH, which appears to be consistent between different automated hematology analyzers. [13] In addition, for HbE carriers and individuals with single α-globin gene deletion (-α3.7 and-α4.2) or non-deletional α-globin gene mutations [i.e., Hb Constant Spring (Hb CS) and Hb Quong Sze], low MCV is not suitable. [14] In addition, the association of heterozygous β-thalassemia with α-thalassemia alone or glucose-6-phosphate dehydrogenase deficiency may result in normal MCV and a false-negative result during thalassemia screening. [15] Thus, screening thalassemia using both MCV and MCH would be more appropriate than just using MCV; it would be very important to determine their cut-off levels using the automated hematology analyzer used in each laboratory.

The interpretation of peripheral blood smear using both MCV and MCH will be regarded as an important form of screening for thalassemia. Microcytosis, hypochromia, and anisopoikilocytosis consist of typical RBC morphology in thalassemia disease. By comparing the size of RBC with those of the nucleus of small lymphocytes, microcytes can be evaluated and hypochromic RBCs are characterised as having an increase in the central pallor diameter of RBCs, that

is, more than one-third of their diameter. Anisopoikilocytosis occurs from different anomalous RBC morphologies including schistocytes, microspherocytes, target cells, polychromasia, and nucleated RBCs. However, only certain forms of thalassemia from other causes of anemia, such as IDA or inflammatory anemia, can be indicated by peripheral blood smear findings, and it is not possible to identify a particular form of thalassemia based solely on RBC morphology. Red cell distribution width (RDW) is a measure of the degree of differences in red cell size, and an increase in RDW is characterised by certain causes of microcytic anaemia, most notably IDA. While thalassemia produces uniform microcytic red cells without a concomitant increase in RDW, this result is variable among the syndromes of thalassemia, including major increases in RDW in HbH disease and minor β-thalassemia. The RDW may also provide details that can be used as an adjunct to the diagnosis, but is not useful as a single screening predictor. [16] As a diagnostic adjunct, the RBC count is often useful because thalassemia causes microcytic anemia with an increase in the number of RBC, but IDA and chronic disease anemia are usually correlated with a decrease in the number of RBC that is proportional to the degree of anemia. However, as a sole screening method for thalassemia and hemoglobinopathies, the RBC count should not be used.

In view of all this, for the screening of thalassemia and hemoglobinopathies, different indices using complete blood count (CBC) components have been established, but none exceed the value of the combination of MCV and MCH in selecting cases for subsequent studies.

## ELECTROPHORESIS AND ADVANCED METHODS

The International Committee for Standardization of Hematology suggested laboratory tests for 3 laboratory forms in 1978. [17] Screening laboratory should be in a position to conduct alkaline electrophoresis in that guideline. Rather complicated experiments such as citrate agar electrophoresis and globin electrophoresis had to be performed by the reference laboratory. These electrophoresis techniques involved manual steps from the preparation of reagents, electrophoresis, and data analysis during the hemoglobin analysis, and thus laboratory professional expertise was a key to successful identification. The launch of updated guidelines was motivated by the recent advancement of laboratory techniques and enhanced awareness of thalassemia and hemoglobinopathy. [18] The presumed identification of hemoglobins is recommended by the British Committee for Standards in Hematology on a minimum of 2 techniques and conclusive identification is known to be based on DNA analysis, mass spectrometry, or protein sequencing.

### High Performance Liquid Chromatography (HPLC):

A tool used to distinguish compounds or molecules based on their chemical properties is the high-performance liquid chromatography (HPLC) technique. Several separation principles are available, such as size, affinity, and partition; ion-exchange chromatography is the most powerful and most commonly used for hemoglobin. The technique can also be manually controlled, but fully automated systems have recently become available. These systems can be used for hemoglobin analysis; however, systems that can switch between glycated diabetes hemoglobin analysis and variant hemoglobin analysis for thalassemia and hemoglobin variants may be more feasible to use in low prevalence areas. It is considered to be useful in the diagnosis of β-thalassemia, since $HbA_2$ can be quantified accurately. [18] Careful monitoring of analytical conditions

such as column temperature, flow rate, and buffer conditions is important, similar to other HPLC techniques.

**Electrophoresis:**

Electrophoresis is a method used in a gel and electrical field to distinguish molecules or compounds based on their migratory pattern. It is also commonly used for protein electrophoresis and differentiation of certain isoenzymes in clinical laboratories. In developed countries, manual preparation of gel and electrophoresis is rarely used as more sophisticated and automated techniques such as capillary electrophoresis are available. Electrophoresis of cellulose acetate is a representative technique of custom electrophoresis. Hb A, F, S / G / D, C / E, and H and other variants are known to allow identification. [18] In many automated HPLC systems, automated capillary electrophoresis is commonly used and has shown benefits in the detection of certain variants that are indistinguishable. [19]

**Mass Spectrometry:**

Based on their mass (molecular weight) to charge ratio, mass spectrometry is a technique to classify molecules. The strong advantage of the technique is that the molecules of interest use limited complex binding reagents. The basic analytical theory makes it possible to define less interference and more accurately. It is not easy to analyse hemoglobin with mass spectrometry because the laboratory should have both technological experience for analysing proteins and a very expensive instrument. Besides recognising hemoglobin based on the molecular weight of the intact molecule, it may also in some degree examine the sequence of amino acids. It is helpful for discovering new variants and checking the sequencing of DNA. [20]

**MOLECULAR CHARACTERIZATION**

In over 90% of cases, α-thalassemia is caused by gene deletion. A minority of cases of α-thalassemia are due to changes in sequence such as single nucleotide substitution, addition, or short addition / deletion. The α gene cluster consists of highly homologous genes and 2 HBA genes which encode identical proteins. Gene deletion is possibly caused during meiosis by unequal crossing between these homologous regions. So far, several breakpoints have been recorded including the most common deletion of 3.7 kb. [21]

About 90% of cases of β-thalassemia are caused by sequence differences relative to cases of α-thalassemia. The β-thalassemia is currently associated with more than 280 sequence variants. [21] Some β-thalassemia is caused by gene deletion which includes the HBB gene.

Numerous different molecular methods are used to identify mutations in the globine gene. Molecular techniques can be classified by mutation type to be targeted as follows: 1) Methods of detection for structural variations, such as gene deletion, duplication or triplication, and 2) Methods of detection for sequence variations, such as nucleotide substitution, insertion or short insertion / deletion. Gap polymerase chain reaction (PCR), specifically designed for the deletion concerned, can detect known gene deletions. For unknown gene deletions, southern blotting using named complementary gene probes can be used. Both known and unknown gene deletions can be identified by the multiplex ligation-dependent probe amplification (MLPA) process. MLPA is commonly used because it is highly sensitive, easy to use and can detect deletions of different kinds.

For certain ethnic groups, typical sequence variations can be identified in a cost-effective manner using techniques such as allele-specific PCR, reverse dot blotting, denaturing gradient gel electrophoresis, and refractory mutation system amplification. Rapid progress and cost

reduction of the sequencing technology made it possible in many laboratories to sequence the globin gene like promoter, 3' UTR, exon-intron boundaries and deep introns. In particular, for targeted genes, exomes, or even genomes, massively parallel sequencing technology can be applied.

## PRENATAL DIAGNOSIS

Prenatal diagnosis involves screening of the carrier, genetic testing and genetic tests of the prenatal gene. To date, the prenatal diagnosis of thalassemia and hemoglobinopathy is one of the world's most commonly conducted genetic analyses. Hemoglobinopathies are widespread in many migrant countries as well as in endemic regions due to population migration. [22-25] The aim of prenatal diagnosis is to recognise and advise asymptomatic individuals whose offspring are at risk of inherited hemoglobinopathy, and to control complications throughout pregnancy. Clinical forms of hemoglobinopathies targeting prenatal diagnosis are associated with potentially serious sequelae and interfere with, for example, sickle cell disease, significant β-thalassemia arising from β-thalassemia homozygosity, and non-immune hydrops fetalis triggered by deletion or malfunction of all 4 α-globin genes. [26] Prenatal diagnosis is beneficial in this case, considering the early lethality of hydrops fetalis, since a large number of women carrying foetuses with this abnormality experience serious toxemia and extreme postpartum hemorrhage. [27] Therefore, a deep understanding of the association between genotype and phenotype, the impact of genetic modifiers and rare cases including dominantly inherited β-thalassemia, uniparental isodisomy and de novo mutation is warranted. [28]

Prenatal diagnosis includes the examination of foetal material in the maternal circulation from chorionic villi, amniotic fluid, cord blood and foetal DNA. Invasive prenatal diagnosis includes the success of first trimester chorionic villus sampling, and second trimester amniocentesis or cordocentesis. While analysis of foetal hemoglobin types is carried out successfully by automated HPLC, it can be determined by examining the foetal blood obtained through cordocentesis, and the procedure is prone to errors due to maternal tissue contamination. [29] In prenatal diagnosis, DNA analysis is particularly useful because irregular hematologic findings are observed and postnatal samples can be collected more easily. The detection of complex thalassemias and hemoglobinopathies seen in ethnically diverse populations has been improved by advances in molecular research. Universal screening services were introduced in Canada and in European countries to identify carriers and give prenatal diagnosis in pregnancies at risk of thalassemia. [30-31] Parental screening is not invasive and can be carried out without risks to the foetus growing. The recent invention of non-invasive prenatal diagnostic testing using maternal plasma cell-free foetal DNA enables active investigation of foetal genetic analysis to avoid invasive procedure. [32] Another benefit of this strategy is that earlier than using an invasive technique, foetal DNA can be separated from the maternal blood. [33] In order to classify foetal hemoglobinopathies, different methods have been used, such as mass spectrometry, next-generation sequencing, and genotyping assay. [34-36] The methods are still challenging, so further research are required to improve and validate them and eventually lead to efficient, accurate , and reliable non-invasive prenatal thalassemia and hemoglobinopathy diagnosis. [37]

## CONCLUSION

Thalassemia and hemoglobinopathies are very common, and the incidence rises as people migrate from endemic areas and marriages become infectious. In addition to family hist-

ory, the doctors should closely study CBC, in particular MCV and MCH, to assume thalassemia and hemoglobinopathies in anemic patients. Using advanced electrophoresis techniques involving HPLC and mass spectrometry, quantitative and structural defects of hemoglobin can be detected more sensitively and precisely. Different molecular tests are used for thalassemia and hemoglobinopathy molecular diagnosis, and recent advances in sequencing technology, such as massively parallel sequencing technology, allow for more accurate genetic diagnosis. Also we should concentrate on prenatal diagnosis and genetic therapy on thalassemia and hemoglobinopathy to avoid births of thalassemia and complication of pregnancy. Disease suspicion by closely examining the screening test results and advanced molecular testing will assist in the early diagnosis and disease intervention. In addition, we expect thalassemia and hemoglobinopathies to establish treatment modality by understanding the molecular and protein characteristics.

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LETTER TO EDITOR

# KEEP HOSPITALS DRY AS MUCH AS MUCH AS POSSIBLE IN ORDER TO PREVENT INFECTIONS

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Unfortunately, nowadays most hospitals around the world are infected [1], and hospital infections kill patients and sometimes even hospital personnel. [2–3] Also, there are too many costs to ward off infections in hospitals in order to prevent antibiotic resistance, and patients are more likely to suffer an infection. Health-care practitioners need a better environment in order to serve patients better, so these measurements are necessary and should be performed unquestionably. However, despite all the efforts, infections are still prevalent in hospitals, and the germs are becoming more resistant to antibiotics. [5–9] An instinctual question is whether there is a simpler solution and eventhough there are probably several solutions, the one that's currently practiced is a thorough disinfection practice of hospitals using different sanitizing products, adding moisture to the dry air. This environment of the hospitals provides the best development habitat for microorganisms, allowing them to develop abundantly. Physicians can transfer germs to patients during an examination; but patients could also spread infections to physicians and other healthcare practitioners. It seems to be better to keep the hospital environment dry as much as possible. However, during hospital transfer or severe cases such as sepsis, it is essential first to use detergent and to ensurethe proper use of specific disinfectants and sterilization methods. It is of great significance to share experience in this regard [10], even though the general public is wellaware of its importance in order to control the spreading of infections. Keeping the hospital environment dry can help prevent and control hospital infections. Of course, the use of disinfectants is highly recommended in some cases where the infection is confirmed by a piece of evidence or if it is likely to cause a hospital-acquired infection and affect the lives of patients. Mainly if microbial resistance is seen in the hospital or among doctors and other healthcare professionals, the use of disinfectants is unavoidable. Physicians, along with the hospital's infection control team must take additional care of patients dealing with infections, the collaboration between them being essential.

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