http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2017.478.488

Review Article
Molecular Modulation of Stress Induced to Abnormal Haematological Indices in Medical Students, Malaysian Perspective

[1]Mohammad Ahmed Issa Al-Hatamleh, [1]Atif Amin Baig, [1]Nordin Bin Simbak, [1]Muhammad Ilyas Nadeem, [2]Saif Ullah Khan and [3]Tengku Mohammad Ariff

[1]Faculty of Medicine, Universiti Sultan Zainal Abidin, Jalan Sultan Mahmud, 20400 Kuala Terengganu, Malaysia
[2]Faculty of Applied Social Sciences, Universiti Sultan Zainal Abidin, Gong Badak, 21300 Kuala Terengganu, Malaysia
[3]Institute for Community Development and Quality of Life, Universiti Sultan Zainal Abidin, Gong Badak, 21300 Kuala Terengganu, Malaysia

Abstract

Medical students in Malaysia and globally were considered among the most exposure groups in the community to stress due to several sources. The most significant and severe sources of stress in medical students is an academic sources, includes a lot of exams/tests, lack of time and facilities for entertainment, stay in a hostel, high parental expectations and vastness of syllabus, in addition to emotional problems and others personal sources. Stress is a very important issue that leads to a worsening of health problems. Stress develops in the body and leads to oxidative stress which in turn leads to a disorder in the whole body. Oxidative stress may lead to abnormal haematological indices elevated white blood cells (WBCs) count. Oxidative stress can lead to massive destruction of red blood cells (RBCs). The brain and the gastrointestinal system (GI) are intimately connected as one system. The brain has a direct impact on the GI tract. A stressful brain can send signals to the gut, just as a troubled intestine can send signals to the brain. Therefore, stress can be the cause of block the breakdown and assimilation of food for energy and nutriment. This malabsorption can then lead to a reciprocal negative effect to the stress and can be another cause of anaemia through malabsorption minerals and vitamins that are to erythropoiesis. So, stress can be one of the leading causes of anaemia among medical students. Stress is a chronic epidemic in the most medical students and can directly affect how well body works. This review article discovers the effect of stress in medical students that can be effect on their studies and further create researcher's interest to generate database that help to reduce stress response and bring about the empowerment of balanced life among Malaysian medical students besides the increasing level of health and academic performance.

Key words: Stress, oxidative stress, free radicals, haematological indices, anaemia

Citation: Mohammad Ahmed Issa Al-Hatamleh, Atif Amin Baig, Nordin Bin Simbak, Muhammad Ilyas Nadeem, Saif Ullah Khan and Tengku Mohammad Ariff, 2017. Molecular modulation of stress induced to abnormal haematological indices in medical students, Malaysian perspective. Pak. J. Biol. Sci., 20: 478-488.

Corresponding Authors: Tengku Mohammad Ariff and Atif Amin Baig, Institute for Community Development and Quality of Life, Faculty of Medicine, Universiti Sultan Zainal Abidin, Gong Badak, 21300 Kuala Terengganu, Malaysia Tel: 6009622-5515, 5646

Copyright: © 2017 Mohammad Ahmed Issa Al-Hatamleh *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

"Hans Selye" defined stress as the common features of a stereo typed reaction of the living organism to all stimuli which tend to disturb the dynamic homeostasis of psychological, biochemical and physiological processes[1]. Antecedent to Selye's were the works of Hippocrates, Bernard and Cannon. Selye's model of stress was essentially a bio-chemical concept[2], he theorized that stress was a result of any non-specific demand made upon an organism. Stress is unavoidable in the life, any person can face it at home, school, work and in our social lives. It is a fact of life and without stress, life would be very boring but on the other hand, too much stress becomes distress, this can become a serious illness if it persists for a long time, especially if it is not treated properly. A person who feels undergoing an untreated stress, may develops psychiatric disorders and can lead to many physiological health problems[3]. Stress occurs when an individual perceives that environmental demands tax or exceed his or her adaptive capacity. Operationally, studies of stress focus either on the occurrence of environmental events that are consensual judged as taxing one's capability to cope or on individual responses to issues that are indicative of this overload[4]. All global studies of stress have been focusing on environmental events causing stress[5]. Study of medicine has been associated with increased exposure of medical students to stress, more than other people in other educational and academic fields. The most important and severe causes of stress in medical students are a lot of exams/tests, lack of time and facilities for entertainment, staying in hostel, high parental expectations and vastness of syllabus, beside emotional problems and others personal sources[6-8]. Socioeconomic status, familial relationships are also producing stress besides daily hassles, exams and academic stress[9]. It has observed, a good social support minimizes the risk of stress[10,11]. Family income with less than 1000 RM has found to be likely more stress in bachelor's degree's students and has a strong association with the mental disorder[12]. Lower socioeconomic status is leading towards stress in students[13]. Students may face problems in their daily expenditure with lower family income that cause poor results. Therefore, medical students in Malaysia are expected to have a few physiological disorders because they have high levels of stress. The medical community needs to know the most prevalent physiological disorders and to confirmation their relationship to stress among medical students in Malaysia.

PREVALENCE OF STRESS AMONG MEDICAL STUDENTS IN MALAYSIA

Faculty of medicines, in Malaysian universities have been reported as a stressful environment, that often exerts a negative effect on the psychological health and consequently on the physical health of the students (Table 1). In a study to explore stress among medical students in Universiti Sains Malaysia (USM), for determining the prevalence, sources and pattern of stress and the factors affecting it, they used a cross-sectional study design utilizing validated questionnaires to evaluate stress levels and stressors among a total of 761 (72%) medical students as respondents in this study. The results concluded the prevalence of stress among the medical students to be 29.6%, reflecting the prevalence of stress among medical students in USM to be very high. Academic related problems were the major stressor among medical students. The academic year of study was the factor most significantly associated with medical student's stress[15]. In another cross-sectional study, conducted in the faculty of medicine at some of local universities in Malaysia, all medical students were selected as respondents. They used an instrument similar to the General Health Questionnaire (GHQ12) to screen for symptoms of psychological stress. The prevalence of psychological stress among medical students was high in this study. As a total of 41.9% of the medical students were found to have psychological stress[16]. Through a cross-sectional study conducted at University Sultan Zainal Abidin (UniSZA), Malaysia, a total of 60 questionnaires were administered to the pre-clinical medical students at faculty of medicine, UniSZA. Study subjects were selected randomly. This study showed that, 47 (78.3%) students might be having stress related/associated problems, several stressful causes have been measured and the major cause of stress was academic because of curriculum overload. Pre-clinical medical students were more stressful, because they were required to follow the fixed schedule. Pre-clinical medical students needed to adhere to stuff, like going to class early every day. Their life style was considered stressful and boring as well as they were just confined to lectures, laboratory and self-study[18]. Academic overload exposes students to stressful situations that was reflected on the level of student performance, lifestyle and health[20]. All mentioned studies indicated an elevated levels of stress which is considered as a chronic stress among medical students in Malaysian universities, especially in the pre-clinical stage. So, this issue required solutions that can help to reduce stress levels in students and improve quality of medical student's life in Malaysian universities.

Table 1: List of stress studies that conducted in Malaysian medical schools

University	Selected students	Prevalence of stress (%)	References
Universiti Putra Malaysia	All medical students	41.9	Sherina *et al.*[14]
Universiti Sains Malaysia	All medical students	29.6	Yusoff *et al.*[15]
Universiti Sains Malaysia	First year medical students	50.0	Yusoff *et al.*[16]
Universiti Putra Malaysia			
Universiti Malaysia Sabah			
Universiti Malaysia Sarawak			
Management and Science University	All medical and medical sciences students	46.0	Al-Dubai *et al.*[17]
Universiti Sultan Zainal Abidin	Preclinical medical students	47.0	Rahman *et al.*[18]
Universiti Sultan Zainal Abidin	Final year medical students	30.56	Rahman *et al.*[19]

HOW THE BODY RESPONDS TO STRESS?

Stress may affect human health through a myriad of behavioral and biochemical pathways due to some key hormonal and metabolic pathways[21]. In this way, chronic stress may influence a variety of physiological disorders through a biochemical cascade[22,23]. Stress starts in the brain and affects the brain as well as the rest of the body. Stress responses support adaptation and survival via responses of neural, cardiovascular, autonomic, immune and metabolic systems[24]. Stress is known leading to stimulate the sympathetic nervous system and hypothalamic pituitary adrenal (HPA) axis, resulting in the release of catecholamines and glucocorticoids[25,26]. Chronic stress may lead to overeating, co-elevation of cortisol and insulin and repression of certain anabolic hormones. This state of metabolic stress leads to increase abdominal fat[27]. The direct stress response and the accumulation of visceral fat both can elevate oxidative stress. This biochemical status conducive to several cell aging mechanisms, mainly inhibiting telomerase and leading to telomere length shortening and cells senescence[28,29]. Oxidative stress is defined as a defect in the balance between output of free radicals and reactive metabolites (antioxidants)[30]. This imbalance can conduct to harm important cells and biomolecules, with possible impact on the whole body[31]. Free radicals are unsettled molecules with electrons that could impair the cell membrane fatty acids and proteins, functioning by reacting with them[32]. Free radicals could be a predisposing factor for a lot of health problems because of their effects on mutation and DNA damage[33]. Free radicals are generated endogenously in human body or exogenously as well, when exposed to different physiochemical conditions or pathological states. Even though a low or moderate reactive oxygen species (ROS) have a good physiochemical effect including the killing of invading pathogens, wound healing and tissue renovation processes[34]. The disproportionate generation of ROS will badly affect homeostasis and causes oxidative tissue damage which is a big serious problem. The reverse impact of ROS can be limited by natural antioxidant pathways but also can be stimulated by many oxidative stressors contributing to tissue damage[35]. ROS are produced in response to exogenous and endogenous agents including stress response. Disorder of normal cellular homeostasis by redox signaling gives a shone in an actual disease for every organ[36]. So, free radicals and antioxidants have become commonly used terms in modern discussions of disease mechanisms[37]. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, NADPH oxidase isoforms (NOX), peroxidases, lipoxygenases (LOXs), xanthine oxidase (XO), glucose oxidase, nitric oxide synthase, myeloperoxidase (MPO) and cyclooxygenase (COX) are all enzymes that catalyze ROS-generating chemical reactions[38,39]. Intracellular compartments including mitochondria, the endoplasmic reticulum, nuclei, peroxisomes, the cytosol, plasma membranes and even extracellular spaces are capable of ROS generation[40,41]. The mitochondrial electron transport chain is the major site of ROS production in most mammalian cells[42]. All the previous studies reported increased oxidative stress and ROS in chronic stress cases[43,44]. Therefore, oxidative stress is a result to several disorders including chronic stress status that can be caused by academic reasons among medical students and this is not surprising. As previously presented, increased oxidative stress in the body causes a harmful increase in ROS, leading to countless physiological disorders.

OXIDATIVE STRESS AND WHITE BLOOD CELLS (WBC)

In chronic stress cases, the elevated levels of oxidative stress are linked to increased levels of ROS in tissues and in blood[45]. ROS are derived from endogenous sources and their production is not neutralized by antioxidant defense mechanisms. Increased levels of ROS production lead to positive-feedback with inflammation related mechanism through pro-inflammatory cytokines trigger ROS production and by ROS induced expression of proinflammatory cytokines[41]. Furthermore, ROS-induced apoptosis of skeletal

muscle fibers is an important contributor to skeletal muscle fatigue and low exercise tolerance[46]. High levels of ROS have been demonstrated in the venous blood of chronic stress cases and are accompanied by high neutrophil superoxide anion generation[47]. Furthermore, the presence of ROS in circulating WBC in chronic stress cases leads to mitochondrial depolarization, which in turn leads to apoptosis of WBC[48]. Elevated WBC count has been shown to have a significant relationship with unfavorable lifestyles such as smoking, obesity, poor sleep and unhealthy diet, which is known to contribute to increase level of stress[49]. A previous study in Japanese male workers marked that poor sleep can be an independent risk factor for an increase in WBC count[50]. The WBC count has also been shown to have an inverse relationship with hour of work, which may have reflected poor sleep[49]. Polymorphonuclear leukocytes (PMNL) are one of the major kinds of inflammatory cells[51]. When the PMNL activated, it release reactive oxygen species, including hydrogen peroxide, contributing to endothelial damage diseases[52,53]. Stress response variables can lead to general fatigue that may be a key determinant of low-grade inflammation as represented by increase neutrophil counts[48]. The chronic secretion of stress hormone corticosterone and stimulation of the adrenal cortical cells with adrenocorticotropic hormone may result in endothelial dysfunction and initiate an acute phase inflammatory response involving the release of cytokines, acute phase proteins and increase neutrophils, decrease monocytes lymphocytes and increases neutrophil-lymphocyte ratio (NLR)[54]. So, chronic stress can lead to elevated total WBCs count due to increased oxidative stress that leads to high levels of harmful ROS.

OXIDATIVE STRESS AND RED BLOOD CELLS (RBC)

The RBCs are constantly risk to meet with both exogenous and endogenous sources of ROS that can harm the RBC and mess up its function[55]. To reduce the impact of the ROS in the oxidative stress status, RBCs have an antioxidant system constituting of both of, non-enzymatic low molecular weight antioxidants like glutathione and ascorbic acid and enzymatic antioxidants including superoxide dismutase, catalase, glutathione peroxidase and peroxiredoxin-2 (PRDX-2)[56-58]. ROS are very interactive and many of the ROS released from macrophages, neutrophils and endothelial cells into the plasma before they can be taken up by RBCs, especially in the microcirculation, because the RBCs more closeness to the blood vessels[59,60]. When the ROS entry into RBC cytoplasm, they are for the most part neutralized by the cytosolic antioxidant system. Hydrogen peroxide attached to RBCs rapidly reacts with catalase being converted to oxygen without any oxidation of haemoglobin (Hb)[55]. Slow autoxidation of Hb generates endogenous ROS with methaemoglobin production which has not the ability to carry oxygen and superoxide production that rapidly dismutases to form hydrogen peroxide[61,62]. The RBC cytosolic antioxidants neutralize the RBC bulk but the antioxidant system to neutralize the endogenous ROS is limited as the blood stream through the microcirculation when Hb becomes partially oxygenated[63]. Partial oxygenation results in an Hb conformational change with certain unique properties. Thus, there is a high increase in the rate of Hb autoxidation for partially oxygenated Hb[62,64]. The excess in the affinity of partially oxygenated Hb for the RBC membrane, limits the efficiency of the antioxidant system from neutralizing the ROS formed at the membrane[65]. This collection of un-neutralized ROS in the RBC leads to damage the RBC membrane impairing the flow of RBCs into the microcirculation and the transfer of oxygen to relevant tissues[66,67]. In addition, recent studies indicated that the RBCs also contain nicotinamide adenine dinucleotide hydrogen (NADH) oxidases, which can generate endogenous ROS[68]. The RBC membrane band 3 is the control of integral trans-membrane protein. It has several crucial functions including: The maintenance of anion homeostasis, thus, providing a link between the membrane and the cytoskeleton accountable for maintaining the cell shape and providing for the reactions of cytosolic proteins with the membrane through the amino terminal region that emerges into the cytosol. This region of band 3 binds competitively to both, Hb and a quantity of glycolytic enzymes[69]. The variations in Hb binding to band 3 as a function of the Hb oxygenation. Thus, couple Hb oxygenation, Hb autoxidation, glycolysis and ATP generation[70]. Oxidative damage to band 3 has been associated with RBC aging including the exposure of senescent specific neo-antigens that connect autologous IgG triggering RBC removal[71]. IgG binding has also been reported to be associated with band 3 clusters, which is triggered by the binding of denatured oxidized Hb (haemichromes) to band 3[72-74]. Caspase-3 activation, which involves oxidative stress, further cleaves the cytoplasmic end of band 3 affecting the communications of band 3 with cytosolic proteins as well as the linkage to ankyrin and the cytoskeleton, which also motivates PS exposure[75,76]. The accelerates of older cells formation is known as membrane micro-vesiculation[77]. These changes affect the highly deformable biconcave shape maintenance which is necessary to pass through narrow pores, thus contributing to their removal from blood circulation, while cell shrinkage and vesiculation can be induced by different factors, some of which may not involve

oxidative stress, the shrinkage related with potassium leakage via the Gardos channel is triggered by oxidative stress[78]. The damage of Ca-ATPase, which maintains a low intracellular concentration of free calcium ions[79]. Damage to Ca-ATPase is accountable for the age induced increase in intracellular calcium and is generated by oxidative harm to the ATPase[80,81]. The increase in the intracellular calcium activates the Gardos channel which leads to potassium leakage from the cell resulting in cell shrinkage and damage[82,83]. This increased also activates calpain, transglutaminase-2 and some caspases that can degrade/crosslink cytoskeleton proteins[84]. It also prevents phosphotyrosine phosphatase rising band 3 phosphorylation[85]. The RBC lipid bilayer contains an asymmetric distribution of phospholipids with PS being maintained on the inner cover of the membrane by the rivalry between scramblase, which randomizes the allocation and flippase, which internalizes the PS. In addition to the increase in sphingomyelinase which increases ceramide, intracellular calcium increase has been linked to the exposure of PS and to the decrease of Flippase activity[86], which triggers the interaction of RBCs with macrophages and eryptosis[87-89]. Despite the important role of macrophages in RBCs removal, it is still not clear whether the RBCs macrophages interaction is the responsible cause of aging RBCs removal from circulation or not[90,91].

OXIDATIVE STRESS AND THE GASTROINTESTINAL (GI) TRACT

The ROS aggressive effect has a tendency to the GI tract, they are also bared to outside environment with immune cells presence and intestinal flora dietary factors, all prospect sources of ROS[92]. Two main enzymatic reactions produce ROS in the GI tract, the hypoxanthine (HX)/XO system and the NADPH oxidase system[93]. The GI tract has the largest concentration of XO in the body, which along with various phagocytic cells and a great number of catalase-negative bacteria in the colon, join to generate large amounts of oxygen (O_2^-)[94]. ROS have been linked with various GI tract disorders includes malabsorption[90]. The excessive levels of ROS can lead to damage cellular proteins including cytoskeletal proteins and ultimately disrupt GI tract barrier to increase gut permeability which contributes to inflammation in a variety of GI tract diseases[95-97]. Therefore, stress can be the cause of block the breakdown and assimilation of food for energy and nutriment. This malabsorption can then lead to a reciprocal negative effect to the stress and can be another cause of many health problems.

OXIDATIVE STRESS AND ANAEMIA

The WHO defines anaemia in adults as haemoglobin less than 130 g L^{-1} in males and less than 120 g L^{-1} in females. Anaemia is one of the most widespread disorders in the world, anaemia affects one-quarter of the world's population and making it a global public health problem, with 1. 62 billion people affected between 1993-2005, approximately 48. 8% of the global population[98]. The cause of anaemia varies with age and sex. Dietary deficiency or malabsorption of haematinic factors like iron or vitamins like folic acid and vitamin B12 are the most important causes of anaemia[99]. Iron deficiency is the most common cause of anaemia by a defect in haemoglobin synthesis. Iron deficiency anaemia affecting about 500 million people worldwide. Moreover, the body has limited ability to absorb iron, Iron is present in food as ferric hydroxides, ferric-protein and heme-protein complexes[100]. Folate and vitamin B12 both are involved in the synthesis of tetrahydrofolate, an integral component of deoxyribonucleic acid synthesis and nuclear maturation. Shortage of vitamin B12 or folate can cause megaloblastic anaemia due to defect accounting for the asynchronous maturation of the nucleus is a defective DNA synthesis that leads to a characteristic abnormality in erythroblasts in the bone marrow (Fig. 1). Vitamin B12 is found in foods of animal origin, such as liver, meat, fish and dairy produce. Vitamin B12 is synthesized in nature by microorganisms by internal production from intestinal bacteria (not in humans) or by eating bacterially contaminated foods. Folate derived from folic acid (vitamin B9) compounds, it's found most especially in foods of animal liver, greens and yeast[100].

Based on the above, oxidative stress resulting in stressful people can lead to GI disorders including malabsorption, which leads to lack of important nutrients to the body including haematinic factors. Also, oxidative stress resulting in stressed individuals plays a role in damaging the RBC membrane and impairing it, or accelerating the aging and death of the RBC. Therefore, stress can be another cause of current health problems including anaemia (Fig. 2) but few studies have been shown the relationship and discussed it (Table 2). In study focusing the of impact psychological stress on serum iron and erythropoiesis in rats by using a communication box system, foot-shock stress and psychological stress were administered to the rats. In the 7th and 14th days after administration, 10 rats were executed, respectively and the femoral bone marrow and blood were collected for analysis of serum iron (SI), serum transferrin receptor (sTfR), serum ferritin (SF), Hb, RBC, red cell

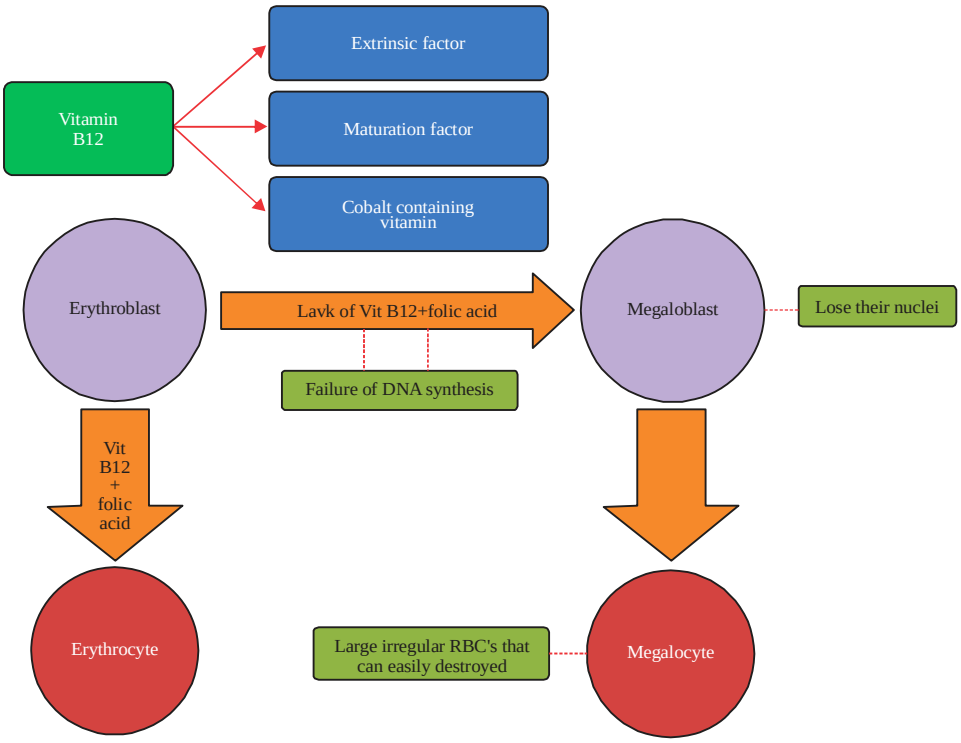

Fig. 1: Role of vitamin B12 and folate in megaloblastic anaemia[101]

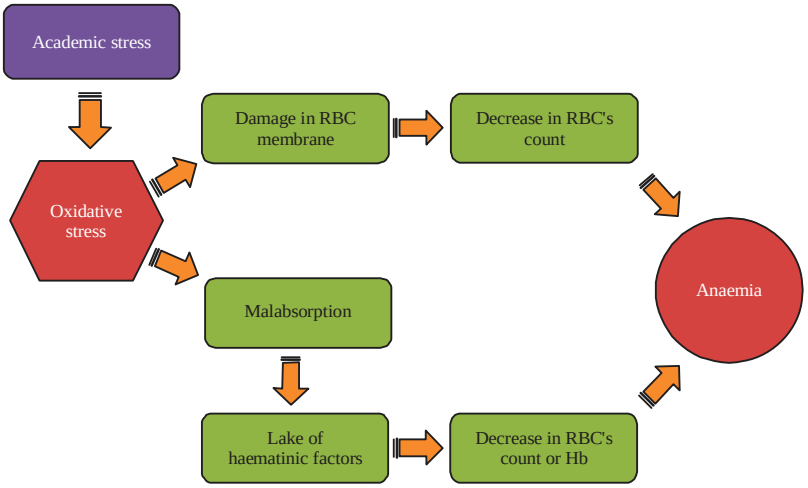

Fig. 2: Mechanism of anaemia caused by academic stress

distribution width (RDW), mean corpuscular volume (MCV), serum erythropoietin (EPO) and bone marrow iron. For rats, analyzed on the 7th and 14th day in psychological stress group, femoral bone marrow iron was significantly reduced, serum iron was reduced, Hb was reduced, RBC count was reduced, MCV was reduced, RDW was increased, serum ferritin, transferrin receptor and EPO showed no significant changes in comparison with controls after 7th day of administration but serum ferritin and EPO were reduced while transferrin receptor increased after 14th day administration. Finally, after comparing the results with a control group after 7th day and 14th day of psychological stress administration concluded, serum iron and bone marrow iron showing significant reducing compared with controls, erythropoiesis was significantly inhibited, all of this leaded to anaemia[103], Another study conducted in Al-Haweeja, Iraq, 200 females aged 20-40 years old (50 women/blood group) to study the prevalence of anemia and relation to psychological

Table 2: List of most important research that linked stress to anaemia

Research samples	Source of stress	Results	References
Females aged 20-40 years old	Deterioration in the quality of life due to general circumstances e.g., economical, political, civil war, recurrent pregnancy and malnutrition	Drop in both Hb and PCV indicates a mild symptomatic anaemia	Hassan *et al.*[102]
Rats	Special communication box system and electric foot-shock	Serum iron and bone marrow iron showed the significant decrease compared with the controls, erythropoiesis was significantly inhibited, all the above led to anaemia	Wei *et al.*[103]

Table 3: List of anaemia studies that conducted in medical colleges

Countries	Universities	Selected students	Prevalence of anaemia (%)	References
Malaysia	Universiti Kebangsaan Malaysia	All medical students	14.5	Azma *et al.*[104]
India	Chhattisgarh Institute of Medical Science	3rd year MBBS students	30.2	Pandey and Singh[105]
India	Sri Guru Ram Das Institute of Medical Sciences and Research	All medical students	29.3	Kaur *et al.*[106]
India	Medical Teaching Institution in Pondicherry	Female medical and nursing students	76.0	Saratha *et al.*[107]
India	Central India	All medical students	39.0	Khan *et al.*[108]
India	Regional Institute of Medical Sciences	All medical students	26.6	Debbarma *et al.*[109]

stress. The study was concluded to complete drop in both Hb and PCV indication a mild symptomatic anaemia, they are suffering mainly due to the deterioration in the quality of life in Al-Haweeja, due to degradation in their general circumstances e.g., economical, financial, political, civil war, recurrent pregnancy and malnutrition that altogether may have caused an inevitable psychological stress leading to anaemia[102].

PREVALENCE OF THE ANAEMIA AMONG MEDICAL STUDENTS

Based on the above-mentioned factors, medical students distinguish them from other groups of society because of additional increased stress levels. So, medical students have a greater chance to be effected by disorders related to stress, including anaemia. There are some studies conducted worldwide to record the prevalence of anaemia among medical students but these findings almost are non-existent in Malaysian medical colleges (Table 3) as per available published data and records.

FUTURE RESEARCH

In last year's there has been a growing appreciation of the issues of quality of life and stress in medical students as this might be affecting their learning and academic performance. Despite the widespread public belief that stress is factor leading to diseases, the biomedical community remains skeptical for this conclusion and need to be further investigated. However, these studies are lacking in medical schools. Authors are looking forward to conduct more studies about this critical issue in Malaysia to assess the prevalence of stress and their relation to increasing exposure to abnormal haematological indices in medical students including anaemia as one of the most prevalent health problems globally. Level of increasing knowledge and confirmation from previous studies, proved and evidenced, how stress can lead to abnormal haematological indices, which requires to be further investigated in the mechanism occurrence of it at molecular and cellular level as per reported and suggested opinions and recommendations. Increase awareness in the community about this problem via health education, preventive intervention with all medical students in Malaysia to reduce stress towards distress, can improve their lifestyle and nutritional status, because it is the significant factor in achieving an improved level of health and academic performance. Further attention must be afforded for this particular issue.

CONCLUSION

Looking at the cited literature, it is hypothesized in the conclusion that there is a correlation between stress in medical students and its effect on the haematological indices include elevated WBCs count, especially neutrophils as well as an increase chance of anaemia that leads to abnormal blood indicators different according to the type of RBCs deficits which led to anaemia. There are several reasons of stress among medical students that needs to more study and there is a need to more investigation in the relationship between oxidative stress and megaloblastic anaemia in medical students in Malaysia.

REFERENCES

1. Burrows, V.L., 2015. The medicalization of stress: Hans selye and the transformation of the postwar medical marketplace. Ph.D. Thesis, University of New York, New York, USA.
2. Landow, M.V., 2006. Stress and Mental Health of College Students. In: Measuring and Understanding Stress in Pharmacy Students, Dutta, A.P., M.A. Pyles and P.A. Miederhoff (Eds.)., Nova Science, New York, pp: 1-28.
3. Fink, G., 2010. Stress Definition and History. In: Stress Science: Neuroendocrinology, Fink, G. (Ed.)., Academic Press, San Diego, pp: 3-44.
4. Cohen, S., D. Janicki-Deverts and G.E. Miller, 2007. Psychological stress and disease. J. Am. Med. Assoc., 298: 1685-1687.
5. Tennant, C., 2002. Life events, stress and depression: A review of recent findings. Aust. N. Z. J. Psychiatry, 36: 173-182.
6. Sreeramareddy, C.T., P.R. Shankar, V.S. Binu, C. Mukhopadhyay, B. Ray and R.G. Menezes, 2007. Psychological morbidity, sources of stress and coping stratergies among undergraduate medical students of Nepal. BMC Med. Educ., Vol. 7. 10.1186/1472-6920-7-26.
7. Abdulghani, H.M., 2008. Stress and depression among medical students: A cross-sectional study at a medical college in Saudi Arabia. Pak. J. Med. Sci., 24: 12-17.
8. Shah, M., S. Hasan, S. Malikand and C.T. Sreeramareddy, 2010. Perceived stress, sources and severity of stress among medical undergraduates in a Pakistani medical school. BMC Med. Edu., Vol. 10. 10.1186/1472-6920-10-2.
9. Brougham, R.R., C.M. Zail, C.M. Mendoza and J.R. Miller, 2009. Stress, sex differences and coping strategies among college students. Curr. Psych., 28: 85-97.
10. Cohen, S. and T. Willis, 1985. Stress social support and the buffering hypothesis. Psychol. Bull., 98: 310-357.
11. Cohen, S. and H.M. Hoberman, 1983. Positive events and social supports as buffers of life change stress. J. Applied Soc. Psychol., 13: 99-125.
12. Everson, S.A., S.C. Maty, J.W. Lynch and G.A. Kaplan, 2002. Epidermiological evidence for the relation between socioeconomic status and depression. Obesity Diabetes. J. Psych. Res., 53: 891-895.
13. Monroe, M.L., P.T. Edward and H.G. Neal, 1980. Childhood depression, family income and locus of control. J. Nervous Mental Dis., 168: 732-735.
14. Sherina, M.S., L. Rampal and N. Kaneson, 2004. Psychological stress among undergraduate medical students. Med. J. Malay, 59: 207-211.
15. Yusoff, M.S.B., A.F.A. Rahim and M.J. Yaacob, 2010. Prevalence and sources of stress among universiti sains malaysia medical students. Malays. J. Med. Sci., 17: 30-37.
16. Yusoff, M.S.B., L.Y. Yee, L.H. Wei, T.C. Siong, L.H. Meng, L.X. Bin and A.F. Abdul Rahim, 2011. A study on stress, stressors and coping strategies among Malaysian medical students. Int. J. Stud. Res., 1: 45-50.
17. Al-Dubai, S.A.R., R.A. Al-Naggar, M.A. AlShagga and K.G. Rampal, 2011. Stress and coping strategies of students in a medical faculty in Malaysia. Malay. J. Med. Sci., 18: 57-64.
18. Rahman, N.I.A., S. Ismail, T.N.A.B.T. Seman, N.F.A.B. Rosli and S.A.B.M. Jusoh *et al*., 2013. Stress among preclinical medical students of University Sultan Zainal Abidin. J. Applied Pharm. Sci., 3: 76-81.
19. Rahman, N.I.A., S. Ismail, R.M. Ali, A.G. Alattraqchi and W.P.E.W. Dali *et al*., 2015. Stress among first batch of MBBS students of Faculty of medicine and health sciences, Universiti Sultan Zainal Abidin, Malaysia: When final professional examination is knocking the door. Int. Med. J., 22: 254-259.
20. Nakalema, G. and J. Ssenyonga, 2014. Academic stress: Its causes and results at a Ugandan university. Afr. J. Teach. Edu., 3: 9-15.
21. Han, K.S., L. Kim and I. Shim, 2012. Stress and sleep disorder. Exp. Neurobio., 21: 141-150.
22. McEwen, B.S., 2008. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. Eur. J. Pharm., 583: 174-185.
23. Gillani, S.W., A.S.S. Syed, O.S. Yelly, S. Azmi, A.B. Atif and B. Mirza, 2011. Perceived stress scale psychometric validation for malaysian diabetic patients. Br. J. Pharm. Res., 1: 156-163.
24. McEwen, B.S., 2008. Brain on stress: How the social environment gets under the skin. Proc. Nat. Acad. Sci., 109: 17180-17185.
25. Rozanski, A. and L.D. Kubzansky, 2005. Psychologic functioning and physical health: A paradigm of flexibility. Psycho Med., 67: S47-S53.
26. Miller, D.B. and J.P. O'Callaghan, 2002. Neuroendocrine aspects of the response to stress. Metabolism, 51: 5-10.
27. Naureen, S., M. Irfan, T. Zin, R. Tariq, A.A. Baig, A. Malik and N. Simbak, 2014. Cortisol and Obesity; A Stress Related Insight: Obesity and Cortisol. Createspace Independent, USA.

28. Correia-Melo, C., G. Hewitt and J.F. Passos, 2014. Telomeres, oxidative stress and inflammatory factors: Partners in cellular senescence? BMC Long Health, Vol. 3. 10.1186/2046-2395-3-1.
29. Aschbacher, K., A. O'Donovan, O.M. Wolkowitz, F.S. Dhabhar, Y. Su and E. Epel, 2013. Good stress, bad stress and oxidative stress: Insights from anticipatory cortisol reactivity. Psychoneuroendocrinology, 38: 1698-1708.
30. Holifa, A., A.Z.A. Latif, N.B. Simbak and A.B. Atif, 2017. Alpha-tocopherol administration in diabetics as preventive and therapeutic agents in oxidative stress. Curr. Tren. Biomed. Eng. Biosci., Vol. 5. 10.19080/CTBEB.2017.05.55567
31. Durackova, Z., 2010. Some current insights into oxidative stress. Physiol. Res., 59: 459-469.
32. Cross, C.E., A. van der Vliet, C.A. O'Neill, S. Louie and B. Halliwell, 1994. Oxidants, antioxidants and respiratory tract lining fluids. Environ. Health Perspect., 102: 185-191.
33. Masaud, I.A., M.A.K. Rohin, A.A. Baig and N. Mohamad, 2014. Potential of therapeutic antioxidant compounds from pomegranate as anti-cancer agent. J. Chem. Pharm. Res., 6: 427-433.
34. Van Wijk, R., E.P. Van Wijk, F.A. Wiegant and J. Ives, 2008. Free radicals and low-level photon emission in human pathogenesis: State of the art. Indian J. Exp. Biol., 46: 273-309.
35. Rao, U.M., M. Haque and A.A. Baig, 2011. Insulin stimulative and anti-oxidative effects of *Persea americana* fruit extract on streptozotocin induced hyperglycemic rats. J. Med. Biol. Sci., Vol. 4.
36. Kim, J. and W. Park, 2014. Oxidative stress response in *Pseudomonas putida*. Applied Microbiol. Biotechnol., 98: 6933-6946.
37. Lobo, V., A. Patil, A. Phatak and N. Chandra, 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn. Rev., 4: 118-126.
38. Kulkarni, A.C., P. Kuppusamy and N. Parinandi, 2007. Oxygen, the lead actor in the pathophysiologic drama: Enactment of the trinity of normoxia, hypoxia and hyperoxia in disease and therapy. Antioxidants Redox Signal., 9: 1717-1730.
39. Swindle, E.J. and D.D. Metcalfe, 2007. The role of reactive oxygen species and nitric oxide in mast cell-dependent inflammatory processes. Immu. Rev., 217: 186-205.
40. Balaban, R.S., S. Nemoto and T. Finkel, 2005. Mitochondria, oxidants, aging. Cell, 120: 483-495.
41. Pritchard, K.A., A.W. Ackerman, E.R. Gross, D.W. Stepp and Y. Shi *et al*., 2001. Heat shock protein 90 mediates the balance of nitric oxide and superoxide anion from endothelial nitric-oxide synthase. J. Biol. Chem., 276: 17621-17624.
42. Poyton, R.O., P.R. Castello, K.A. Ball, D.K. Woo and N. Pan, 2009. Mitochondria and hypoxic signaling: A new view. Ann. New York Acad. Sci., 1177: 48-56.
43. Salim, S., 2014. Oxidative stress and psychological disorders. Curr. Neuropharmacol., 12: 140-147.
44. Rahman, K., 2007. Studies on free radicals, antioxidants and co-factors. Clin. Interv. Aging, 2: 219-236.
45. Nita, M. and A. Grzybowski, 2016. The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxid. Med. Cell Long., Vol. 23. 10.1155/2016/3164734.
46. Powers, S.K., L.L. Ji, A.N. Kavazis and M.J. Jackson, 2011. Reactive oxygen species: Impact on skeletal muscle. Compr. Physiol., 1: 941-969.
47. Mittal, M., M.R. Siddiqui, K. Tran, S.P. Reddy and A.B. Malik, 2014. Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signall., 20: 1126-1167.
48. Nishitani, N. and H. Sakakibara, 2014. Association of psychological stress response of fatigue with white blood cell count in male daytime workers. Indus. Health, 52: 531-534.
49. Nakanishi, N., K. Suzuki and K. Tatara, 2003. Association between lifestyle and white blood cell count: A study of Japanese male office workers. Occup. Med., 53: 135-137.
50. Nishitani, N. and H. Sakakibara, 2007. Subjective poor sleep and white blood cell count in male Japanese workers. Ind. Health, 45: 296-300.
51. Baggiolini, M., U. Bretz, B. Dewald and M.E. Feigenson, 1978. The polymorphonuclear leukocyte. Inflam. Res., 8: 3-10.
52. Smedly, L.A., M.G. Tonnesen, R.A. Sandhaus, C. Haslett and L.A. Guthrie *et al*., 1986. Neutrophil-mediated injury to endothelial cells: Enhancement by endotoxin and essential role of neutrophil elastase. J. Clin. Inves., 77: 1233-1243.
53. Weiss, S.J., 1989. Tissue destruction by neutrophils. N. Engl. J. Med., 320: 365-376.
54. Dhabhar, F.S., W.B. Malarkey, E. Neriand and B.S. McEwen, 2012. Stress-induced redistribution of immune cells-from barracks to boulevards to battlefields: A tale of three hormones-curt Richter award winner. Psychoneuroendocrinology, 37: 1345-1368.
55. Mohanty, J.G., E. Nagababu and J.M. Rifkind, 2014. Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. Fron. Phys., Vol. 5. 10.3389/fphys.2014.00084.
56. Nagababu, E., J.G. Mohanty, J.S. Friedman and J.M. Rifkind, 2013. Role of peroxiredoxin-2 in protecting RBCs from hydrogen peroxide-induced oxidative stress. F. Rad. Res., 47: 164-171.
57. Nagababu, E., F.J. Chrest and J.M. Rifkind, 2003. Hydrogen-peroxide-induced heme degradation in red blood cells: The protective roles of catalase and glutathione peroxidase. Biochim. Biophys. Acta, 1620: 211-217.
58. Lee, T.H., S.U. Kim, S.L. Yu, S.H. Kim and D.S. Park *et al*., 2003. Peroxiredoxin II is essential for sustaining life span of erythrocytes in mice. Blood, 101: 5033-5038.

59. Nagababu, E. and J.M. Rifkind, 1998. Formation of fluorescent heme degradation products during the oxidation of hemoglobin by hydrogen peroxide. Biochem. Biophys. Res. Commun., 247: 592-596.
60. Rifkind, J.M., R.S. Ajmani and J. Heim, 1997. Impaired hemorheology in the aged associated with oxidative stress. Adv. Exp. Med. Biol., 428: 7-13.
61. Aoshiba, K., Y. Nakajima, S. Yasui, J. Tamaoki and A. Nagai, 1999. Red blood cells inhibit apoptosis of human neutrophils. Blood, 93: 4006-4010.
62. Abugo, O. and M. Rifkind, 1994. Oxidation of hemoglobin and the enhancement produced by nitrobluetetrazolium. J. Biol. Chem., 269: 24845-24853.
63. Pandey, K.B. and S.I. Rizvi, 2011. Biomarkers of oxidative stress in red blood cells. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub., 155: 131-136.
64. Balagopalakrishna, C., P.T. Manoharan, O.O. Abugo and J.M. Rifkind, 1996. Production of superoxide from hemoglobin-bound oxygen under hypoxic conditions. Biochemistry, 35: 6393-6398.
65. Cao, Z., J.B. Bell, J.G. Mohanty, E. Nagababu and J.M. Rifkind, 2009. Nitrite enhances RBC hypoxic ATP synthesis and the release of ATP into the vasculature: A new mechanism for nitrite-induced vasodilation. Am. J. Physiol.-Heart Circulat. Physiol., 297: H1494-H1503.
66. Nagababu, E., S. Gulyani, C.J. Earley, R.G. Cutler, M.P. Mattson and J.M. Rifkind, 2008. Iron-deficiency anaemia enhances red blood cell oxidative stress. F. Rad. Res., 42: 824-829.
67. Barodka, V.M., E. Nagababu, J.G. Mohanty, D. Nyhan, D.E. Berkowitz, J.M. Rifkind and J.J. Strouse, 2014. New insights provided by a comparison of impaired deformability with erythrocyte oxidative stress for sickle cell disease. Blood Cells Mol. Dis., 52: 230-235.
68. George, A., S. Pushkaran, D.G. Konstantinidis, S. Koochaki and P. Malik *et al*., 2003. Erythrocyte NADPH oxidase activity modulated by Rac GTPases, PKC and plasma cytokines contributes to oxidative stress in sickle cell disease. Blood, 121: 2099-2107.
69. Mohandas, N. and P.G. Gallagher, 2008. Red cell membrane: Past, present and future. Blood, 112: 3939-3948.
70. De Rosa, M.C., A.C. Carelli, A. Galtieri, A. Russo and B. Giardina, 2008. Allosteric properties of hemoglobin and the plasma membrane of the erythrocyte: New insights in gas transport and metabolic modulation. IUBMB Life, 60: 87-93.
71. Kay, M.M., 1993. Generation of senescent cell antigen on old cells initiates IgG binding to a neoantigen. Cell. Mol. Biol., 39: 131-153.
72. Low, P.S., S.M.Waugh, K. Zinke and D. Drenckhahn, 1985. The role of hemoglobin denaturation and band 3 clustering in red blood cell aging. Science, 227: 531-533.
73. Rettig, M.P., P.S. Low, J.A. Gimm, N. Mohandas, J. Wang and J.A. Christian, 1999. Evaluation of biochemical changes during *in vivo* erythrocyte senescence in the dog. Blood, 93: 376-384.
74. Ferru, E., K. Giger, A. Pantaleo, E. Campanella and J. Grey *et al*., 2011. Regulation of membrane-cytoskeletal interactions by tyrosine phosphorylation of erythrocyte band 3. Blood, 117: 5998-6006.
75. Mandal, D., V. Baudin-Creuza, A. Bhattacharyya, S. Pathak, J. Delaunay, M. Kundu and J. Basu, 2003. Caspase 3-mediated proteolysis of the N-terminal cytoplasmic domain of the human erythroid anion exchanger 1 (band 3). J. Biol. Chem., 278: 52551-52558.
76. Grey, J.L., G.C. Kodippili, K. Simon and P.S. Low, 2012. Identification of contact sites between ankyrin and band 3 in the human erythrocyte membrane Biochemistry, 51: 6838-6348.
77. Willekens, F.L., J.M. Werre, Y.A. Groenen Dopp, B. Roerdinkholder Stoelwinder, B. De Pauw and G.J. Bosman, 2008. Erythrocyte vesiculation: A self protective mechanism? Br. J. Haematol., 141: 549-556.
78. Rifkind, J.M. and E. Nagababu, 2013. Hemoglobin redox reactions and red blood cell aging. Antioxidants Redox Signal., 18: 2274-2283.
79. Larsen, F.L., S. Katz and B.D. Roufogalis, 1981. Calmodulin regulation of Ca^{2+} transport in human erythrocytes. Biochem. J., 200: 185-191.
80. Samaja, M., A. Rubinacci, R. Motterlini, A. De Ponti and N. Portinaro, 1990. Red cell aging and active calcium transport. Exp. Gerontol., 25: 279-286.
81. Kiefer, C.R. and L.M. Snyder, 2000. Oxidation and erythrocyte senescence. Curr. Opin. Hematol., 7: 113-116.
82. Foller, M., R.S. Kasinathan, S. Koka, C. Lang and E. Shumilina *et al*., 2008. TRPC6 contributes to the Ca^{2+} leak of human erythrocytes. Cell. Physiol. Biochem., 21: 183-192.
83. Brugnara, C., 1993. Membrane transport of Na and K and cell dehydration in sickle erythrocytes. Experientia, 49: 100-109.
84. Redding, G.S., D.M. Record and B.U. Raess, 1991. Calcium-stressed erythrocyte membrane structure and function for assessing glipizide effects on transglutaminase activation. Proc. Soc. Exp. Biol. Med., 196: 76-82.
85. Zipser, Y., A. Piade, A. Barbul, R. Korenstein and N.S. Kosower, 2002. Ca^{2+} promotes erythrocyte band 3 tyrosine phosphorylation via dissociation of phosphotyrosine phosphatase from band 3. Biochem. J., 368: 137-144.
86. Burger, P., E. Kostova, E. Bloem, P. Hilarius Stokman and A.B. Meijer *et al*., 2013. Potassium leakage primes stored erythrocytes for phosphatidylserine exposure and shedding of pro coagulant vesicles. Br. J. Haematol., 160: 377-386.
87. Daleke, D.L., 2008. Regulation of phospholipid asymmetry in the erythrocyte membrane. Curr. Opin. Hematol., 15: 191-195.
88. Foller, M., S.M. Huber and F. Lang, 2008. Erythrocyte programmed cell death. IUBMB Life, 60: 661-668.
89. Weiss, E., D.C. Rees and J.S. Gibson, 2010. Role of calcium in phosphatidylserine externalisation in red blood cells from sickle cell patients. Anemia, 10.1155/2011/379894.

90. Dasgupta, S.K., H. Abdel-Monem, P. Guchhait, S. Nagata and P. Thiagarajan, 2008. Role of lactadherin in the clearance of phosphatidylserine-expressing red blood cell in sickle cell disease. Transfusion, 48: 2370-2376.
91. Saxena, R.K., N. Bhardwaj, S. Sachar, N. Puri and S. Khandelwal, 2012. A double *in vivo* biotinylation technique for objective assessment of aging and clearance of mouse erythrocytes in blood circulation. Transf. Med. Hemother., 39: 335-341.
92. Muresan, A., S. Suciu, D.R. Mitrea, C. Alb, C. Login and D. Crisan, 2008. Oxidative stress implications in experimental gastric ulcer induced by indomethacin. Bull. UASVM. Vet. Med., 65: 119-125.
93. Bhattacharyya, A., R. Chattopadhyay, S. Mitra and S.E. Crowe, 2014. Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol. Rev., 94: 329-354.
94. Mastroeni, P., 2002. Immunity to systemic Salmonella infections. Curr. Mol. Med., 2: 393-406.
95. Phull, P.S., C.J. Green and M.R. Jacyna, 1995. A radical view of the stomach: The role of oxygen-derived free radicals and anti-oxidants in gastroduodenal disease. Eur. J. Gastroenterol. Hepatol., 7: 265-274.
96. Rao, R., R.D. Baker and S.S. Baker, 1999. Inhibition of oxidant-induced barrier disruption and protein tyrosine phosphorylation in Caco-2 cell monolayers by epidermal growth factor. Biochem. Pharm., 57: 685-695.
97. Banan, A., S. Choudhary, Y. Zhang, J.Z. Fields and A. Keshavarzian, 1999. Ethanol-induced barrier dysfunction and its prevention by growth factors in human intestinal monolayers: Evidence for oxidative and cytoskeletal mechanisms. J. Pharmacol. Exp. Ther., 291: 1075-1085.
98. McLean, E., M. Cogswell, I. Egli, D. Wojdyla and B. de Benoist, 2009. Worldwide prevalence of anaemia, WHO vitamin and mineral nutrition information system, 1993-2005. Public Health Nutr., 12: 444-445.
99. Thakur, N., N. Rai and A.F. Siddiqui, 2016. Nutrition in Anemia. In: Handbook of Nutrition and Diet in Leukemia and Blood Disease Therapy, Watson, R.R. and D. Mahadevan (Eds.)., Wageningen Acad. Publ., Wageningen, pp: 353-370.
100. Hoffbrand, A.V. and P.A. Moss, 2016. Hoffbrand's Essential Haematology. 7th Edn., John Wiley and Sons, Oxford.
101. Snow, C.F., 1999. Laboratory diagnosis of vitamin B12 and folate deficiency: A guide for the primary care physician. Arch. Int. Med., 159: 1289-1298.
102. Hassan, Z.A., N.A. Chelebi, A.A. Bazzaz and S.A. Bazzaz, 2016. Correlation of psychological stress to severity of anemia in Al-Haweeja women. Eur. J. Pharm. Med. Res., 3: 248-251.
103. Wei, C., J. Zhou, M. Li and X. Huang, 2008. Effects of psychological stress on serum iron and erythropoiesis. Int. J. Hematol., 88: 52-56.
104. Azma, R.Z., O. Ainoon, I. Azlin, H. Hamenuddin, N.A. Hadi and W.K. Tatt, 2012. Prevalence of iron deficiency anaemia and thalassaemia trait among undergraduate medical students. Clin. Terap., 163: 287-291.
105. Pandey, S. and A. Singh, 2013. A cross sectional study of nutritional Anemia among medical students in a medical college, at Bilaspur, Chhattisgarh. Nat. J. Med. Res., 3: 143-146.
106. Kaur, M., A. Singh, R. Bassi and H. Kaur, 2015. Nutritional status and anaemia in medical students of Sgrdimsar, Amritsar. Nat. J. Physiol. Pharm. Pharmacol., 5: 45-49.
107. Saratha, A., Z. Singh, S.S. Datta, A.V. Boratne, V. Senthilvel and S. Joice, 2010. Prevalence of anaemia among young adult female students in a medical teaching institution in Pondicherry. Indian J. Matern. Child Health, 12: 1-8.
108. Khan, B., N.D. Sukhsohale and P. Jawade, 2015. Prevalence of anemia among undergraduate medical students of Central India. Global J. Res. Anal., 4: 13-14.
109. Debbarma, R., P. Paul, B. Debbarma, T. Sutnga and B. Loukrakpam, 2016. Anaemia among medical students of Regional Institute of Medical Sciences (RIMS), Imphal. Sch. J. Applied Med. Sci., 4: 3744-3748.