

Original Article

Effects of Yoga in Type 2 Diabetes Mellitus With Hypertension : Alteration in RBC Morphology as a Marker for Oxidative Stress

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Abstract

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Purpose: Yoga is well known for improving oxygenation to the biological system and combating oxidative stress which is responsible for numerous lifestyle diseases which includes type 2 diabetes and hypertension. Therefore, the present study was conducted to evaluate the effects of yoga in modifying and improving the quality of life in type II diabetic with hypertension patients.

Methods: An interventional, prospective and open labeled study was done involving 30 patients of type 2 diabetes along with hypertension. Patients received yoga therapy for 45 days along with the standard treatment. Oxidative stress markers such as changes in RBC morphology (crenated edges and Heinz bodies), malondialdehyde levels along with fasting blood glucose levels, systolic & diastolic blood pressure, body mass index and symptoms associated with type 2 diabetes were evaluated before and at the end of the yoga therapy.

Results: Abnormal RBCs were markedly reduced as according to the severity ranking assessed after 45 days of yoga therapy. Significant reduction in the levels of malondialdehyde ($P < 0.01$), blood glucose ($P < 0.05$), Systolic blood pressure ($P < 0.01$) body mass index ($P < 0.001$) and improvement in the unpleasant symptoms were observed after yoga therapy when compared to same patients before starting yoga therapy.

Conclusion: These findings suggest that yoga intervention has therapeutic values in patients having type 2 diabetes with hypertension. This may have direct impact on the dose minimization of hypoglycemic drugs of the patient which requires further study in this area.

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Introduction

Stress can be described as a circumstance that disturbs the normal psychological or physiological functioning of a person¹. Stress can be acute or chronic that causes stimulation of hypothalamic pituitary adrenal axis (HPA axis), sympathetic nervous system and also increases the production of reactive oxygen species (ROS) followed by release of stress hormones (2). The stress hormones especially adrenaline, glucagon, corticosteroid cause lipolysis, neoglucogenesis and increase in blood pressure which leads to metabolic syndrome (2, 3, 13, 14). ROS causes cell damage and increase the synthesis of proinflammatory and inflammatory mediators like interleukin-1, tumor necrosis factor and prostaglandins. The isoprostanes 8-iso PGF₂ alpha are formed non-enzymatically from arachidonic acid directly by free radicals. This isoprostanes binds to the prostanoid receptors and cause chronic inflammation (3, 6, 8, 9, 24). When the balance between pro-oxidant and anti-oxidants is deranged due to excessive production of free radicals or low level of anti oxidants, the state is called Oxidative Stress (7).

This is responsible for insulin resistance, RBC and tissue damage in type II diabetes (3, 4). RBCs are the first cells to be affected by ROS and chronic inflammation causing damage to the cell membrane (crenated edges) and haemoglobin (heinz bodies) (2, 3, 4). The damaged RBCs are removed from the circulation by the spleen during the life span. Therefore, RBC morphology is used as a biomarker for oxidative stress in Type II diabetes.

The free radicals can also damage unsaturated fatty acids in cell membrane. Plasma lipoproteins leads to the formation of lipid peroxides and highly reactive dialdehydes that can chemically modify the proteins and nucleic acid bases. The total body radical burden can be measured from the products of lipid peroxidation (6). Malondialdehyde (MDA) is one of the most toxic byproducts of lipid peroxidation which is of major toxicological interest. The toxic byproducts formed during lipid peroxidation have effects at site away from the area of their generation hence they behave as toxic secondary messengers (9).

Yoga is an ancient discipline designed to balance physical, mental, emotional and spiritual well being in an individual. Yoga therapy has gained popularity nowadays because of its unique nature of delivering positive improvement towards numerous disorders as well as subsiding the disease progression. It includes gentle stretching of muscles and breathing exercises with wide range of classical asanas and pranayama practices. Many studies proved the pathogenic role of oxidative stress in lifelong disorders which necessitates this study to be involved with type 2 diabetes along with hypertensive patients. The aim of the study is to evaluate the impact of yoga therapy on the RBC morphology with relevance to oxidative stress. This may provide us the better understanding the molecular mechanism of how yoga therapy involved in reversing the free radical induced damages in RBC morphology.

Methodology

It was an interventional, prospective and open labeled study. The present study involved 30 Patients of either sex between the age group of 40 to 60 years old diagnosed with type 2 diabetes mellitus as well as hypertension undergoing treatment for more than 5 years as outpatients were recruited for the study after explaining the complete study purpose and procedures. Recruitment of the patients was done after Institutional Ethics committee approval (No.41102015). Informed consent was obtained from the patients who were willing to participate in the study in the prescribed format in regional language. If the patient was illiterate, left thumb impression in the presence of an impartial witness was taken. The demographic details of the patients were obtained and recorded. History of the patients was taken. Pregnant and lactating women, physically handicapped or mentally ill, patients with any advanced complications of diabetes (retinopathy and nephropathy) and those who are already practicing yoga were excluded from the study. As there was no control group included in this study because of the longer treatment status of the patients. The general & systemic examinations were carried out. 3 ml of blood was collected and transferred to EDTA coated tubes. It was centrifuged at 2000 rpm for 10 minutes at 4°C. The top yellow plasma layer was

pipetted off without disturbing the white buffy layer. The plasma was stored on ice and then transferred to a deep freezer at -80°C for estimation of the MDA levels later. The packed cells were reconstituted as 10% v/v suspension with 0.9% normal saline. A drop of this suspension was put on a glass slide under a cover slip and studied under high power microscope for assessment of morphological changes in the red blood cells.

Morphological changes in RBC's at baseline and after 45 days of yoga therapy were assessed using the following scoring pattern (4) :

- No abnormal RBC/HPF = 0
- 10-25% abnormal RBC/HPF = 1+
- 25-50% abnormal RBC/HPF = 2+
- 50-75% abnormal RBC/HPF = 3+
- >75% abnormal RBC/HPF = 4+

Presence of crenated edges with Heinz bodies in RBCs were considered as abnormal RBCs. Malondialdehyde was estimated by using the thiobarbituric acid reactive substances (TBARS) assay kit which was purchased from Caymen Chemicals, USA. The fasting blood glucose levels, body mass index, systolic and diastolic blood pressure were measured and the assessment of improvement of symptoms was done using the questionnaire as shown in the Table I.

Intervention with yoga schedule:

The yogasana schedule was designed by naturopathist involves the combination of asanas and breathing exer-cises. All the patients were trained in order to the follow the yoga schedule for 45 days. Yoga schedule starts with OM chanting (5 min) followed by naadi suthi prayanama as well as ujjai prayanama (5 min) and various asanas includes Ardha Halasana, Naukasana, Ushtrasan, Ardha pawanmuktasana, Salabasana, vakrasana, Bhujangasan, Chakrasana, katicasana and Shavasan (20 min). These asanas are selective according to the Patient condition. They were recommended to practice these asanas twice a day. They were also handed over a booklet regarding the same. If they found any difficulty in performing those asanas or if they felt any pain or injury while performing any asana, the particular asana was modified by the yoga specialist. Patients practicing yoga were asked to report once in 15 days to ensure that they were practicing the yogasana schedule regularly and that they had no difficulty in performing the asanas. The subjects were allowed to withdraw from the study at any point, if they so desired. Statistical analysis was done using Paired t test.

Results

Among 30 patients in which 6 patients were withdrawn from the study due to the reason irrelevant to yoga practice. The variation was observed in the regularity pattern among the patients. Out of 24 patients, 14 were male and 10 were female with

TABLE I: Questionnaires for assessing the improvement in diabetic complications.

S.No.	Symptoms	The symptom was _____ troublesome to me.				
		A (not at all)	B (little)	C (moderate)	D (very)	E (Extremely)
1	Lack of energy					
2	Urinary frequency					
3	Aching Intensity in Calves					
4	Dry mouth					
5	Thirst frequency					
6	Existence of irritability before meal					
7	Feeling of Numbness in Palm and feet					
8	Palpitation Frequency					
9	Sense of Fatigue					

The patients are alphabetically graded according to the severity of symptoms.

Mean±SD age of 53±9 years. RBC Morphology was improved as the damage induced by the free radical significantly declined after the yoga therapy was confirmed through the scoring patterns (Fig. 1 & 2).

Significant reduction in the levels of malondialdehyde (<0.01), fasting blood glucose (<0.05), systolic blood pressure (<0.01) and body mass index (<0.001) was observed after the yoga therapy as shown in the Table II.

The diabetic complications were progressively decreased after the yoga therapy and the significant

improvement in their well being patterns was assessed through the validated self-made questionnaire (31) (Table III). Few patients even reported that they reduced the frequency of taking the hypoglycemic medications themselves, still they found good glycemic control. Data of those few patients has not been shown separately as it is beyond the scope of this study.

Discussion

As a result of chronic stress, sympathetic nervous system (SNS) activation causes the release of

TABLE II: Represents the changes in the parameters before and after the 45 days of yoga therapy.

S. No.	Parameter	Before	After	P value
1	Malondialdehyde (µM/L)	64.95±14.97	47.25±18.50	<0.01
2	Blood glucose Levels (mg/dl)	200.96±78.01	137.26±53.15	<0.05
3	Systolic blood pressure (mmHg)	141.4±10.04	135.66±9.14	<0.01
4	Diastolic blood pressure (mmHg)	93.8±12.25	89.3±7.90	0.2117
5	Body Mass Index (BMI)	23.24±2.68	22.85±2.66	<0.001

Values are expressed in Mean±SD. P value shows significant for malondialdehyde levels (0.01), blood glucose levels (0.05), systolic blood pressure (0.01) and body mass index (0.001).

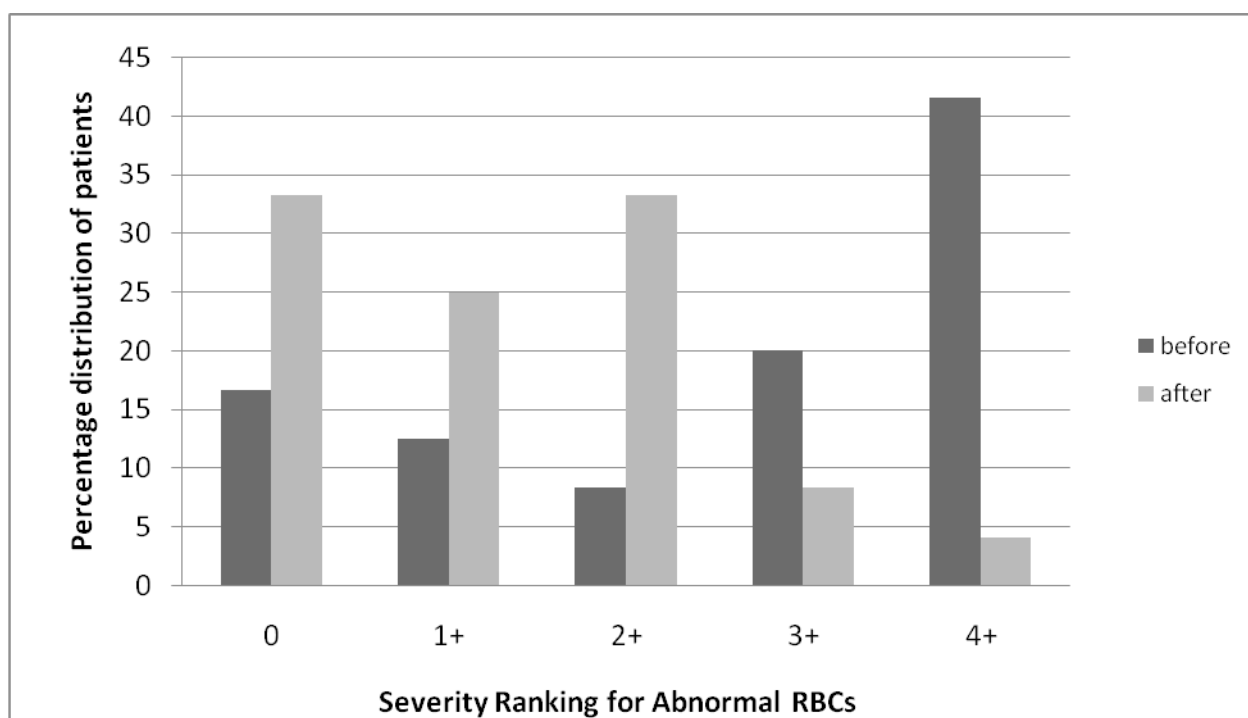


Fig. 1: Represents the change in the severity ranking of the RBC Morphology before and after the 45 days of Yoga therapy.

TABLE III: Percentage distribution of number of patients based on symptoms severity before and at the end of 45 days of yoga therapy.

S. No.	Symptoms questionnaires	Percentage Distribution of number of patients based on the severity (%)									
		A		B		C		D		E	
		Before	After	Before	After	Before	After	Before	After	Before	After
1	Lack of energy	0	29	4	58	17	13	54	0	25	0
2	Urinary frequency	0	29	8	50	25	21	63	0	4	0
3	Aching Intensity in calves	16	17	21	33	50	17	29	0	16	0
4	Intensity of Dry mouth	27	54	15	38	27	8	31	0	0	0
5	Thirst frequency	8	29	12	46	29	25	38	0	13	0
6	Existence of Irritability before the meal	79	87	0	13	0	0	17	0	4	0
7	Feeling of Numbness or Loss of sensation in feet	29	42	12	29	25	21	17	8	17	0
8	Palpitation Frequency	46	71	0	27	29	4	17	0	8	0
9	Sense of Fatigue	50	54	17	29	21	17	0	12	0	0

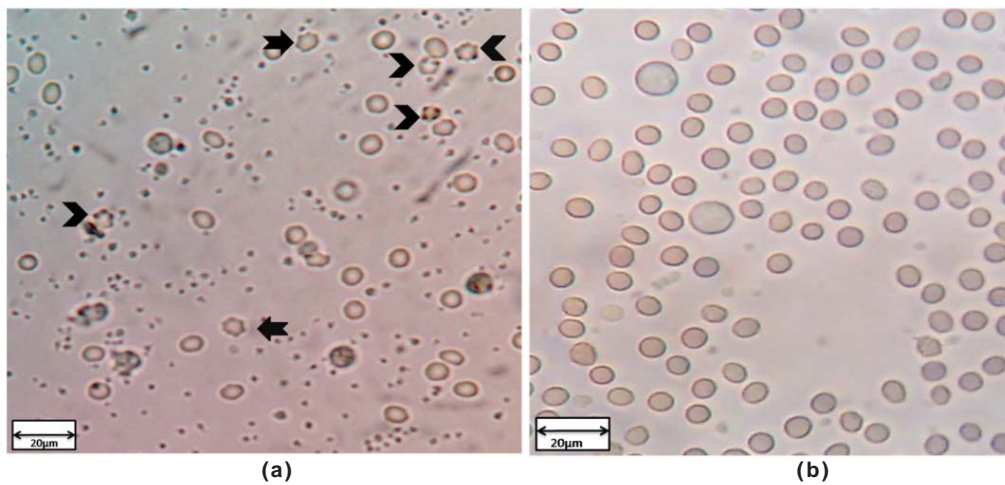


Fig. 2: Shows the optical microscopy images (40x) of (a) the Heinz bodies along with crenated edges present in RBCs (b) the Normal RBCs.

➡ Indicates Crenated edges present in RBCs. ➤ Indicates Heinz bodies present in RBCs.

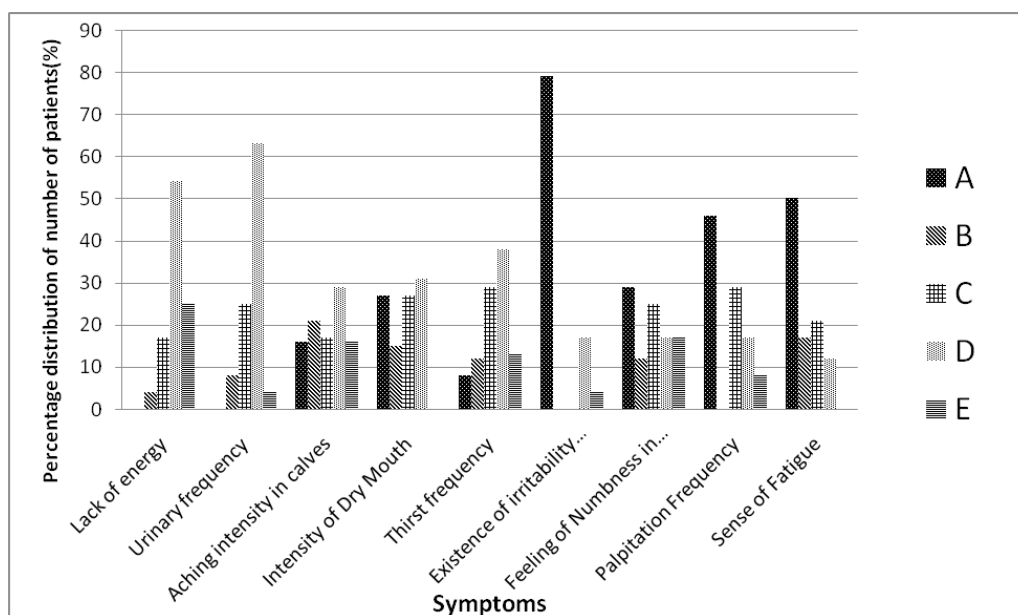


Fig. 1(a): Represents the intensity of symptoms before the yoga therapy.

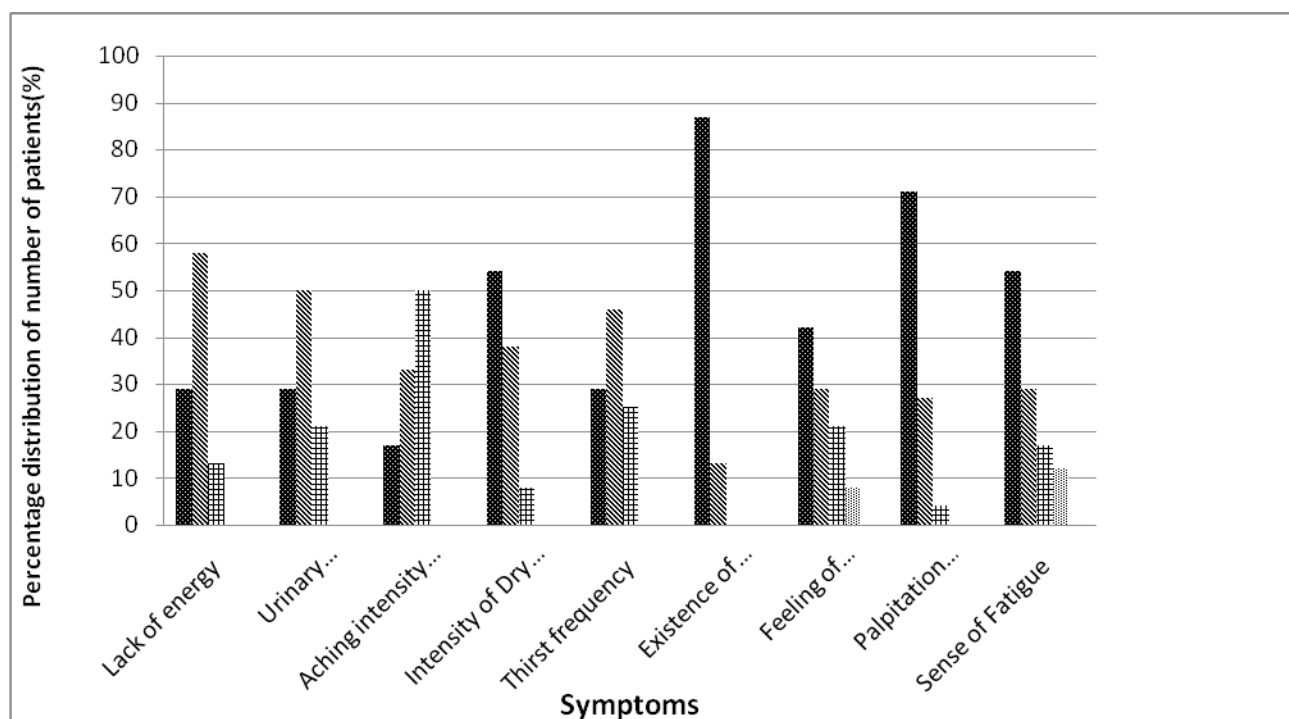


Fig. 2(b): Represents the intensity of the symptoms after the 45 days of yoga therapy.

Noradrenaline and Epinephrine which leads to increase in heart rate, force of contraction and increased peripheral vascular resistance. In addition, SNS stimulates the release of renin which in turn increases Angiotensin II and Aldosterone secretion causing sodium and water retention. The overall effect on cardiovascular system (CVS) contributes to the increased blood pressure (11).

Low blood glucose level due to fasting is the normal stimulus for Glucagon. During periods of stress, trauma or severe exercise, the increased release of Adrenaline, stimulates the secretion of Glucagon even in euglycemic state, in anticipation of increased glucose use (11). Adrenaline and Glucagon stimulate gluconeogenesis from glycogen store in liver and adipose tissue. Adrenaline suppresses the release of insulin, while glucagon antagonizes the effect of insulin, resulting in severe hyperglycemia. Glycogenolysis, gluconeogenesis and decreases utilization of glucose in muscle and adipose tissue causes Insulin Resistance (13). There is increased release of other anti-insulin hormones like corticosteroids, growth hormone which causes neoglucogenesis, decrease peripheral utilization of

glucose leading to persistent hyperglycemia (14). In diabetic patients, gluconeogenesis is induced by stress hormones (Adrenaline and Glucagon) contributes to hyperglycemia. Hyperglycemia leads to changes in osmolarity of body fluids, intracellular acidosis and increased production of free radicals (ROS) (8, 9, 10).

RBCs are highly susceptible to free radical damage due to high concentration of oxygen and haemoglobin. Normal mature RBC lacks mitochondria therefore it is completely dependent on glycolysis for production of ATP which is required to meet the metabolic needs of RBCs. The circulating RBCs have effective antioxidant systems like reduced glutathione pool to protect the cell from oxidative damage. Glutathione also helps to maintain reduced state of sulphhydryl groups in protein and haemoglobin in RBCs. In case of oxidative stress conditions like diabetes, the glutathione pool gets depleted exposing the RBCs to oxidative stress. Pyruvate kinase converts phosphoenol pyruvate to pyruvate. This is third irreversible reaction of glycolysis producing ATP (adenosine triphosphate). RBCs are completely dependent on this reaction for the production of ATP due to lack of

mitochondria. Decreased ATP Production causes alteration in RBC cell membrane leading to changes in the shape and flexibility of RBC's (crenated edges).

Oxidation of sulphhydryl groups in protein including haemoglobin forms denatured proteins that forms insoluble masses of haemoglobin called Heinz bodies that attach to cell membrane. This alteration in RBCs leads to premature death and lysis resulting in haemolytic anaemia (2). The circulating inflammatory mediators like prostanoids (PGF₂α) which are synthesized non enzymatically due to ROS also causes damage to RBC cell membrane and haemoglobin.

Yoga therapy which induces asanas and prayanama relieves mental stress, increase blood flow and oxygenation to all the tissues. This reduces the sympathetic over activity, release of stress hormones,

production of reactive oxygen species and the synthesis of inflammatory mediators. Therefore, yoga therapy decreases insulin resistance, control hyperglycemia and correction of haemolytic anaemia.

Conclusion

It can be concluded from this study that Regular yoga practice is very effective in minimizing the oxidative stress induced damage in RBC morphology and also beneficial in improving glycemic parameter, blood pressure and body mass index with potential to minimize disease complication. This may have direct impact on the dose reduction of hypoglycemic and hypotensive drugs of the patient which requires further study. These findings suggest that yoga Intervention has certain therapeutic value in Patients with type 2 diabetes with hypertension by reducing the symptoms of the disease status effectively.

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