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Original Article

Effect of pranayam practicing on lipid peroxidation and antioxidants in coronary artery disease.

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ABSTRACT

Yoga is a greatest Indian concept annunciated to the world. Accumulating evidences indicates that incidence and progression of coronary artery disease (CAD) may be modified by yoga. Free radicals play an important role in pathogenesis of tissue damage in many clinical disorders. Oxidative stress was assessed by estimating lipid peroxidation [LPO]. Activity of enzymatic antioxidants superoxide dismutase [SOD], glutathione peroxidase [GPx], catalase. The levels of heart profile enzymes creatine kinase (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) were measured in 60 patients of CAD ranging in age 40-60 years. Levels of plasma LPO, CK-MB, LDH, and AST were Significantly raised but activity of SOD, GPx, catalase, were significantly lowered in CAD on comparing with control groups. The study strongly indicates that after practicing of group of five pranayam *(Bhastrika Pranayam, Kapalbhati Pranayam, Bhya Pranayam, Anulom-vilom Pranayam, Brahmari Pranayam)* there was significantly increased activity of SOD, GPx and catalase. The study suggests that practicing of these five pranayama with drug therapy increases the activity of antioxidant enzymes and reduces the lipid peroxidation and heart profile enzymes which prevents cellular damage due to free radicals in CAD

1. Introduction

Coronary artery disease (CAD) remains the major cause of morbidity and mortality in all developed countries in the world as well as in India [1]. The cost management of CAD is significance economic burden and so prevention of CAD is very important. Prevention of CAD can be approached by various ways including health probation campaigns, specific probation strategies, life style modification programs, diet modification, early detection, good control to risk factors and vigilance of emerging risk factors [2].

In recent years, accumulating evidences indicates that incidence and progression of CAD may be modified by yoga. Yoga is a greatest Indian concept annunciated to the world. The popularization of yoga in the West by Yoga School influenced by the Yoga Sutras of Patanjali (2nd century BC). The origin of yoga is in the Vedic Shastra, which is the foundation of Indian tradition. For civilized society and for a successful individual, yoga is perhaps the finest and most clearly laid out uplifting system available so far [3]. Pran or vital life energy can mean several things breath, life, consciousness, air, energy, etc. All the actions carried out by our body are directly or indirectly related to pran. Generally pran is used in various ways to represent the life giving air or breath for life, pranayama is compound world combining pran and ayam. Thus pranayam means controlling of breath. There are number of pranayam types. The pranayama practiced in this study are as fallows,

Bhastrika Pranayam: In this sit in any comfortable position and inhale till diaphragm is full and exhale with force, this is known as Bhastrika pranayam and should be done according to individual capacity in three different ways, slow, medium and fast. This pranayam should be practiced for three to five minutes. While practicing this you should think that the divine strength energy, purity, calmness and happiness prevailing in the universe are positive and that vital life energy is entering inside our body. When you inhale contract the stomach and fill the air till the diaphragm. This will prevent the stomach and will remain in chest and ribs [3].

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Kapalbhati Pranayam: Kapal means brain and bhati means light or shine or brightness. This is slightly different from Bhastrika pranayam. In this inhaling, exhaling is done with the same speed but in Kapalbhati force is laid on exhaling with full force. In this do not try to inhale but air enters automatically while exhaling. The exhaling process should be with full concentration. While practicing this pranayam we should think that air that we are breathing out is taking away with it all the diseases present in the body. If we think that our body is getting rid of the diseases and positive energy is entering then it has positive impact. This pranayam should be practiced for five minutes [3].

Bhya Pranayam: In this sit in Padmasan and exhale all at once completely with full force then do Moolbandh, Uddiyan and Jalandhar bandh and control the breath outside for a long possible. Then remove all three bandh and breathe normally. Inhale and repeat the pranayam without stopping it up to 3-7 times according to capacity. We should always think positive while doing this pranayam and think that all the diseases and dosha present in the body are going away and the body is being filled with fresh new positive energy. This type of auspicious and positive attitude gives beneficial results [3].

Anulom-vilom Pranayam: This is one of the most important pranayam that helps us to bring balance between the distance of the dominance of left and right hemispheres of the human brain. In this press right nostril with right thumb and breath in completely from left nostril then close the left nostril with middle and ring finger and exhale completely from right nostril. Then inhale from right nostril and exhale from left nostril. The pace of breathing should be increased from medium and then fast. Inhale with full strength and breathe out in the same manner. This completes one cycle. This rhythmic breathing should be repeated viz. inhale from left nostril and exhale from right nostril and vice-versa[3].

Brahmari Pranayam: Inhale and press the nostrils with the tips of middle fingers and concentrate in between the two eyes. Close both ears with thumbs and make a sound like Om. Repeat it once again and do at least 3-21 times according to capacity. This pranayam should be done with full concentration and deep devotion towards God. We should think mentally that God is bestowing his grace, happiness and peace on us. The God appears in the form of a divine light and will take away all the ignorance present in the mind and make me wise. In this way, the pranayam should be done with pure fillings and the practitioner will be able to medicate automatically[3]. These indicated five pranayam techniques were adopted in this study.

Free radicals play an important role in pathogenesis of tissue damage in many clinical disorders [4]. Normally there is balance between tissue oxidant and antioxidant activity. Latter is achieved by the antioxidant system, which indicates enzymes SOD, catalase, GPx and antioxidant vitamin C, Vitamin A, vitamin E and other carotenoids [5]. Recent study have shown that yoga can lower systemic blood pressure, decrease resting heart rate, decrease nor adrenaline production in brain and other tissues and have an indirect effect on metabolism of glucose, lipids and neurotransmitters. All these factors have an important role in the pathogenesis of CAD [3].

Considering these facts we planned to study the effects of these five pranayam *(Bhastrika Pranayam, Kapalbhati Pranayam, Bhya Pranayam, Anulom-vilom Pranayam, Brahmari Pranayam)* practicing for protection of heart in coronary heart disease with the help of investigating lipid peroxidation, antioxidant enzymes such as SOD, GPx ,Catalase and heart profile enzymes CK-MB, LDH and AST.

2. Materials and Methods

2.1. Study Design

The study group was consisting of 60 coronary artery disease patients in age group 40-60 years. Out of 60 CAD patients 30 were kept on practicing of these five pranayam and 30 were kept on drug therapy. In this study age and sex matched 60 normal healthy subjects from Anand Yoga Center, Nipani and Belgaum were used as controls. All the patients in this study were selected from Out Patient Department (OPD) District hospital, Belgaum and clinically diagnosed with the help of clinical, physiological, biochemical and pathological tests. The study was approved from the ethical committee of the institute and written consent was obtained from patients.

2.2.Exclusive Criteria

The patients associated with renal disease, liver disease, lung disease, thyroid disease, gastrointestinal disease, alcoholic, tobacco chewers and smokers etc. that could alter the required parameters were excluded from the study. The CAD patients included in this study were excluded from history drugs therapy and life style practice (aerobic exercises).

2.3.Sample Collection

The fasting venous blood samples obtained from these subjects were used for the analysis. The buffy coat was removed and the packed cells were washed three times with physiological saline.

2.4. Analytical Methods

A detail clinical history was taken and all the routine investigations including Hb, TLC, DLC, and blood urea and serum creatinine were carried out. In addition lipid profile, MDA and antioxidant enzymes and heart profile enzymes were estimated in all the cases. Standard procedures were followed for various estimations- serum total cholesterol [6], serum triacylglycerol [7], serum LDL [8], and serum HDL [9]. The erythrocyte suspension was prepared by the method of Dodge et al [10], modified by Quist [11]. The packed cells were used for the analysis of MDA, SOD, catalase and GPx. MDA was determined as the measure of thiobarbituric acid reactive substances (TBARS) [12]. SOD activity was determined in the hemolysate by the method of Mishra and Fridovich based on the inhibition of autoxidation of epinephrine to adenochrome at pH 10.2 [13]. Catalase activity was measured by the method of Beer and Seazer [14] and GPx activity by Paglia and Valentine in erythrocytes [15]. The activity of CK-MB was determined by immunoinhibition method, kinetic assay [16] and AST by modified UV (IFCC) kinetic assay [17]. The LDH activity was measured by kinetic assay [18].

2.5.Pranayam Practicing

The patients of CAD were kept on practicing of these five pranayama regularly one hour at early morning 5.00 am to 6.00 am and one hour at evening 6.00 pm to 7.00 pm in fresh air. The pranayam practiced by patients were Bhastrika breathing- 10 min. (20 breaths in two rounds), Kapalbhati pranayama- 40 min. (5 minuets 1 round, 8 rounds), Anulom-Vilom pranayama-30 min. (5 minuets 1 round, 6 rounds) Bhya pranayama and Brahmari were practiced for 7 times.

At the end of 4, 6 and 8 weeks practicing of five pranayam the changes in biochemical parameters were taken. The lipid profile, lipid peroxidation and antioxidants were performed initially before practicing pranayama before starting drug therapy. These were repeated after 4, 6 and 8 weeks practice of pranayama and drug therapy in CAD.

All the measurements were carried out by using Microsoft office 'Excel' with Windows 2007 operating system and all the data were analyzed using the statistical package SPSS-10.0.

3. Result

Table1. Lipid peroxidation , antioxidant enzymes and heart profile enzymes before and after pranayama practicing in coronary artery disease.

	Zone of inhibition (mm)				
Parameters	**Healthy Controls (n=60) Mean ± SD**	**CAD Before Pranayama Practicing (n=30) Mean ± SD**	**CAD After 4 weeks Pranayama Practicing (n=30) Mean ± SD**	**CAD After 6 weeks Pranayama Practicing (n=30) Mean ± SD**	**CADAfter 8 weeks Pranayama Practicing (n=30) Mean ± SD**
MDA (n moles/gm of Hb)	3.20± 0.68	5.57± 0.88	5.00± 0.55 **	4.45± 0.30 *	3.98± 0.27***
SOD (IU/gm of Hb)	1866.60± 522.65	1450.20± 325.50	1570.00± 338.32**	1602.28± 350.18 *	1682.82 ±412.01***
Catalase (nmole/H_2O_2 decomposed mg protein/min)	645±110.10	428±95.88	453 ±95.85**	488±102.05*	528±109.90***
GPx (IU/gm of Hb)	55.55± 5.58	38.40± 3.99	42.24± 3.99**	48.48± 4.12 *	59.58± 4.18***
CK-MB (IU/L)	18.20 ±6.87	65.7 ±4.4	55.3 ±6.46	41.0 ±8.03	34.10 ±4.53
LDH (IU/ L)	380 ±20.07	644.5 ±55.47	596.0 ±32.30	560 ±25.25	480 ±30.03
AST (IU/L)	20.25 ±8.32	56.9 ±10.0	49 ±12.10	43.45 ±8.0	31.4 ±9.30

n = Number of observations. **indicates p < 0.0001 compared with CAD before Pranayama Practicing. *indicates p < 0.001 compared with after 4 weeks pranayama Practicing. ***indicates p < 0.01 compared with after 6 weeks pranayama Practicing.

Table 2. - Lipid peroxidation, antioxidant enzymes and heart profile enzymes before and after drug therapy in coronary artery disease.

	Zone of inhibition (mm)				
Parameters	**Healthy Controls (n=60) Mean ± SD**	**CAD Before Pranayama Practicing (n=30) Mean ± SD**	**CAD After 4 weeks Pranayama Practicing (n=30) Mean ± SD**	**CAD After 6 weeks Pranayama Practicing (n=30) Mean ± SD**	**CADAfter 8 weeks Pranayama Practicing (n=30) Mean ± SD**
MDA (n moles/gm of Hb)	3.20± 0.68	5.38± 0.53	5.02± 0.68 **	4.25± 0.25 *	3.30± 0.30***
SOD (IU/gm of Hb)	1866.60± 522.65	1410.18± 321.11	1550.00± 350**	1625.20± 345*	1705±250***
Catalase (nmole/H_2O_2 decomposed mg protein/min)	645±110.10	435±98.22	450 ±92**	480±100.05*	560 ±35***
GPx (IU/gm of Hb)	55.55± 5.58	40.40± 3.99	40 ± 3.33**	46.50 ± 5.48	50 ± 3.95***
CK-MB (IU/L)	18.20 ±6.87	65.7 ±4.4	55.3 ±6.46	41.0 ±8.03	34.10 ±4.53
LDH (IU/ L)	380 ±20.07	644.5 ±55.47	596.0 ±32.30	560 ±25.25	480 ±30.03
AST (IU/L)	20.25 ±8.32	56.9 ±10.0	49 ±12.10	43.45 ±8.0	31.4 ±9.30

n = Number of observations. **indicates p < 0.0001 compared with CAD before drugs therapy. *indicates p < 0.001 compared with after 4 weeks drugs therapy. n = n

Table 3. Levels of serum lipid profile before and after pranayama practicing in coronary artery disease.

	Zone of inhibition (mm)				
Parameters	**Healthy Controls (n=60) Mean ± SD**	**CAD Before Pranayama Practicing (n=30) Mean ± SD**	**CAD After 4 weeks Pranayama Practicing (n=30) Mean ± SD**	**CAD After 6 weeks Pranayama Practicing (n=30) Mean ± SD**	**CADAfter 8 weeks Pranayama Practicing (n=30) Mean ± SD**
Total Cholesterol (mg/dl)	162 ± 25.45	293 ± 30.02	220 ±25.30*	195±30.18**	175±22.52***
Triacylglycerol (mg/dl)	105.50 ±35.38	260 ±61.50	200 ±42.65*	172.73±35.5**	125.22±30.02***
LDL (mg/dl)	96.75 ±23.20	189 ±37.7	135 ±28.12*	130±25.50**	110±19.90***
HDL (mg/dl)	49.40 ±9.08	25 ±8.87	39.82 ±6.78*	42.38±7.67**	45.40±8.55***

n = Number of observations. **indicates $p < 0.0001$ compared with CAD before Pranayama Practicing. *indicates $p < 0.001$ compared with after 4 weeks pranayama Practicing. ***indicates $p < 0.01$ compared with after 6 weeks pranayama Practicing.

Table 4. - Levels of serum lipid profile before and after drugs therapy in coronary artery disease.

	Zone of inhibition (mm)				
Parameters	**Healthy Controls (n=60) Mean ± SD**	**CAD Before Pranayama Practicing (n=30) Mean ± SD**	**CAD After 4 weeks Pranayama Practicing (n=30) Mean ± SD**	**CAD After 6 weeks Pranayama Practicing (n=30) Mean ± SD**	**CADAfter 8 weeks Pranayama Practicing (n=30) Mean ± SD**
Total Cholesterol (mg/dl)	162 ± 25.45	350 ± 32	250 ±30*	202±31.38**	170±32.20***
Triacylglycerol (mg/dl)	105.50 ±35.38	295 ±55	1550.00± 350**	1625.20± 345*	1705±250***
LDL (mg/dl)	96.75 ±23.20	238 ±35.37	148 ±32*	129±19.28**	100±17.78***
HDL (mg/dl)	49.40 ±9.08	22.97 ±7.77	35 ±7*	40±4.53**	47.65±6.86***

n = Number of observations. **indicates $p < 0.0001$ compared with CAD before drugs therapy. *indicates $p < 0.001$ compared with after 4 weeks drugs therapy. ***indicates $p < 0.01$ compared with after 6 weeks drugs therapy.

4. Discussion

In coronary artery disease there was statistically significant increase in the levels of erythrocyte MDA. The activity of erythrocyte antioxidant enzyme SOD, GPx and catalase were significantly decreased in CAD when compared with controls.

The lipid peroxidation product i.e. MDA levels have been increased (Table 1 and Table 2). These results are on par with findings of K. Kour et al and Cavalca V. et al [18,19]. MDA is used as an index of oxidative damage [19]. The high levels of MDA indicate increased membrane lipid peroxidation. The enhanced lipid peroxidation may occur as a result of suppression of scavenging mechanisms and increased the free radical generation processes [20]. It has been suggested that hypercholesterolemia, can cause an increase in lipid peroxidation [21]. In present study hypercholesterolemia is observed (Table 3 and Table 4), this may cause increase in lipid peroxidation. After practicing of these five pranayam for 4 and 6 weeks there was significant decrease in the levels of cholesterol and it resumes to normal after 8 weeks. The HDL levels were increased and LDL levels were decreased after practicing of these five pranayam. This increased HDL may be responsible for lowering total cholesterol levels in CAD after five pranayam practicing.

In CAD patients there was significantly decreased activity of enzyme SOD. Similar results have been shown by Handan AK. et al and Landmessar UIF et al [21,22]. SOD is the important antioxidant enzyme having scavenging effect against superoxide anion. In CAD there is hypoxia, reperfusion and increased concentration of LDL, this cause's increased production of superoxide anions [23]. The rate of secretion of TNF-α in CAD is increased and it contributes to depression of extra-cellular SOD activity [22]. Thus activity of SOD may be decreased in CAD. Hence due to increased superoxide anions and secretion of TNF-α, activity of SOD may be lowered in CAD.

In CAD patients there was significantly decreased activity of catalase and GPx. These results are similar to Kharb [24]. Catalase is responsible for detoxification of H_2O_2 produced by action of superoxide dismutase and inhibits the formation of superoxide radical [25]. It contains tetrameric subunit of protein. Increased oxidative stress may oxidize tetrameric subunit of catalase by reactive free radicals. This results in loss of activity of catalase in CAD. Increased oxygen free radicals may reduce activity of glucose-6-phosphate dehydrogenase, results in decreased NADPH generation. This reduced NADPH lowers conversion of oxidized glutathione to reduced glutathione, thus there is progressive loss of reduced glutathione. Therefore the activity of glutathione peroxidase was decreased in CAD patients.

The lowered activity of SOD, GPx and catalase were significantly raised after practicing 4 and 6 weeks of these five pranayama and it comes within normal range after 8 weeks. After 8 weeks drugs therapy reduced activity of these antioxidant enzymes were comes to normal.

In CAD patients there was significantly increased levels enzymes CK-MB, LDH-1 and AST. After practicing of these five pranayam and drugs therapy these raised levels were significantly decreased and come to normal range.

5. Conclusion

In conclusion, the present study strongly advocates that after practicing of group of five pranayam *(Bhastrika Pranayam, Kapalbhati Pranayam, Bhya Pranayam, Anulom-vilom Pranayam, Brahmari Pranayam)* there was significantly increased activity of antioxidant enzymes SOD, GPx and catalase and significantly decreased activity of CK-MB, LDH and AST. This study indicates that practicing of these five pranayama increase activity of antioxidant enzymes and reduces the levels of lipid peroxidation, which protects cellular damage due to free radicals in CAD. Thus study strongly suggests that with drug therapy, practicing of these five pranayam may be curing CAD in short period.

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