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Oxidative Stress Status in Hypertensive Patients on Capoten Treatment

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Abstract: ***Background: hypertension is a condition in which the blood vessels have persistently raised pressure, putting them under increased stress. Oxidative stress is an imbalance between ROS and antioxidant defense mechanisms, causing damage to biological macromolecules and dysregulation of normal metabolism and physiology. Oxidative stress is contributes to the etiology of hypertension in humans. Capoten is an angiotensin-converting enzyme (ACE) inhibitor. Which is responsible for the conversion of angiotensin I to angiotensin II. The aim of this study: was to assess the oxidative stress in hypertensive patients on Capoten treatment through the assessment of salivary Malondialdehyde (MDA) and superoxide dismutase (SOD) as a marker of oxidative stress. Material and method: 60 individuals were included in this study, divided into two groups; one study group and one control group. The first group composed of 30 hypertensive patients on Capoten antihypertensive agent. The second group (control group) composed of 30 healthy subjects without any systemic disorder and almost healthy oral hygiene. Intraoral examination was done for each individual. Saliva samples were collected in restful and quit circumstances, the salivary flow rate (F/R) was calculated ml per minute. PH of salivary secretion were measured by PH meter. The levels of salivary MDA and SOD were analyzed by using ELISA kit based on the principle of Competitive enzyme immunoassay technique, the concentrations of markers were measured by spectrophotometer at 450nm in a microplate reader. Results: salivary MDA was significantly higher in patients groups in relation to control group. Salivary SOD was significantly lower in patient groups in relation to control group. Salivary flow rate and PH was significantly lower in patient groups comparing to control group. Conclusions: salivary MDA and SOD can be used as potential marker for monitoring patients with Hypertension. There is a relation between oxidative stress and hypertension.***

Keywords: Oxidative stress, hypertension, Capoten, MDA, SOD, Salivary flow rate and PH

1. Introduction

Hypertension is defined as a systolic blood pressure (SBP) of 140 mm Hg or more, or a diastolic blood pressure (DBP) of 90 mm Hg or more, or taking antihypertensive medication.(1) Hypertension may be primary, which may develop as a result of environmental or genetic causes, or secondary, which has multiple etiologies, including renal, vascular, and endocrine causes.[(2)] Primary or essential hypertension accounts in 90-95% of adult cases, and secondary hypertension accounts for 2-10% of cases.[(3)]

Capoten (Captopril) is an angiotensin converting enzyme (ACE) inhibitor.[(4)] Capoten prevents the conversion of angiotensin I to angiotensin II which is a potent endogenous vasoconstrictor substance, also stimulates aldosterone secretion from the adrenal cortex, contributing to sodium and fluid retention.[(5)] Oxidative Stress (OS) is an imbalance between the generation of reactive oxygen species (ROS) and nitrogen species (RNS) and the antioxidant defense systems in the body.[(6)] Under normal conditions, ROS and the byproducts of their reactions with various biomolecules are neutralized and converted to harmless molecules by the natural antioxidant system. The antioxidant defense system is a highly complex biochemical organization that consists of numerous enzymes and a large number of scavenger molecules, the body's pool of antioxidant molecules is derived from endogenous and exogenous sources.[(7)] superoxide dismutase (SOD), have been identified as an endogenous antioxidant enzyme.[(8)] Reactive O2- is converted by SOD into H2O2. In the next step, H2O2 is converted into H2O and O2 by salivary enzymes, catalase, peroxidase, and glutathione peroxidase.[(9)] The main primary products of lipid peroxidation are lipid hydroperoxides (LOOH). Among the many different aldehydes which can be formed as secondary products during lipid peroxidation, malondialdehyde (MDA), propanol, hexanal, and 4-hydroxynonenal (4- HNE).[(10)] MDA appears to be the most mutagenic product of lipid peroxidation.[(11)] MDA is an end-product generated by decomposition of arachidonic acid and larger Polyunsaturated fatty acids (PUFAs).[(12)] Once formed MDA can be enzymatically metabolized or can react on cellular and tissue proteins or DNA to form adducts resulting in biomolecular damages.[(12)] MDA is one of the most popular and reliable markers that determine oxidative stress in clinical situations.[(13)] OS contributes to the etiology of hypertension in humans,[(14)] also Hypertensive patients have impaired endogenous and exogenous antioxidant defense mechanisms.[(15)]

2. Subject, Material and Method

Sixteen individuals were included in this study, divided into two groups; one study groups and one control group. The first group composed of 30 hypertensive patients on capoten treatment with mean age 55.10 year (±3.166 SD); 20 were males (67%) and 10 were females (33%).

The second group (control group) composed of 30 healthy subjects without any systemic disorder and almost healthy oral hygiene with mean age 54.77 year (±3.339 SD); 15 males (50%) and 15 females (50%). After explaining the experimental design and the purpose of the study written informed consent was signed from each patient participate in this study. All patients were selected from Al-Manathera Primary Health Center in AL- Najaf city. After gathering

information regarding age, sex, the dose of medication per day, family history of hypertension, oral soft tissue condition, burning mouth syndrome if existed, signs and symptoms of dry mouth, salivary flow rate, PH of saliva, saliva sample for laboratory analysis were collected from all the selected individuals under the similar conditions.

Methods: Intraoral examination was done for each individual using sterile dental mirror and probe with artificial light. The examination was performed systemically in the following sequence:

- Oral mucosa, examination of oral soft tissues was done in a sequence according to W.H.O. (1997).
- Burning mouth syndrome according to (Scala, et al., 2003).
- Signs and symptoms of dry mouth.

The same dentist performed all examinations. A concordant diagnostic analysis was performed on 12 randomly selected patients by a second examiner.

Saliva samples were collected in restful and quit circumstances. Following flushing of mouth with distal water. Saliva produced during the first 2 minutes was discarded to avoid any possible contamination, spitting saliva into graduated test tubes. After the collection of adequate amounts of saliva (5 ml) according to the biological needs, the salivary F/R was calculated ml per minute. PH of salivary secretion were measured by PH meter. Salivary samples were centrifuged at 3000×rpm for 15 minutes at-80, and then the clear supernatant was taken and transported frozen in ice crushed container to the laboratory and stored at -80C until analysis.

1) Estimation of Salivary Superoxide Dismutase

The level of salivary superoxide dismutase was analyzed by using commercially available, BG SOD ELISA kit. It is based on the principle of Competitive enzyme immunoassay technique utilizing a monoclonal anti-SOD antibody and an SOD-Horseradish Peroxidase (HRP) conjugate.

The assay sample and buffer are incubated together with SOD-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the SOD concentration, since SOD from samples and SOD-HRP conjugate compete for the anti-SOD antibody binding site. A standard curve is plotted relating the intensity of the color Optical Density (O.D.) to the concentration of standards. The SOD concentration in each sample is interpolated from this standard curve.

2) Estimation of Salivary Malondialdehyde

The level of salivary malondialdehyde (MDA) will be analyzed by using commercially available, BG MDA ELISA kit. It is based on the principle of competitive enzyme immunoassay technique utilizing a monoclonal anti-MDA antibody and an MDA -Horseradish Peroxidase (HRP) conjugate.

The assay sample and buffer are incubated together with MDA-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplatereader. The intensity of the color is inversely proportional to the MAD concentration since MAD from samples and MAD-HRP conjugate compete for the anti-MAD antibody binding site. Since the number of sites is limited, as more sites are occupied by MAD from the sample, fewer sites are left to bind MAD-HRP conjugate. A standard curve is plotted relating the intensity of the color Optical Density (O.D.) to the concentration of standards. The MAD concentration in each sample is interpolated from this standard curve.

Statistical analysis: Data were translated into a computerized database structure. An expert statistical advice was sought for study. Statistical analysis were computer assisted using SPSS version 24 (Statistical Package for Social Sciences). In association with Excel version 5. The results were expressed as Mean±Standard Deviation (SD). The differences between the groups were analyzed by using the Student's "t"-test and one way ANOVA with the post hoc Tukey test and Pearson's correlation was applied to determine the relationships between the variables. The statistical significance was defined at a p value of <0.05.

3. Results

Age & Gender

A total of sixteen individuals were participated in this study, divided into two groups (one study groups of and one control group). The study group were 30 hypertensive patients on Capoten with a mean age of 55.10 year (±3.166 SD); 20 were males (67%) and 10 were females (33%). The age range was (50-60) years.

Regarding control group, 30 of healthy subjects without any systemic disorder and almost good oral hygiene were included in this study. The mean age was 54.77 year (±3.339 SD); 15 were males and 15 were females. The age range was (50-63) years, Table 1.

Table 1: The age range, mean and standard deviation of the study group and control

Groups	N	Age Range (years)	Mean age (year)	±SD	P-value
Control	30	50-63	54.77	3.339	P>0.01
Amlodipine	30	52-60	56.53	2.161	

**N= number*

As shown in **Table 1**, statistically no significant differences in mean age were found between patient and control group (P>0.01).

Salivary flow rate (F/R) & PH.: Both salivary flow rate & PH of hypertensive patients found to be significantly lower than that of the control group. As shown in **Table2.**

Table 2: Salivary Flow-rate and PH in Study and Control Group

Variables	N	Mean	±SD	Std. Error	P-value
Salivary flow rate (ml/min)	Control (n=30)	0.37	0.14	0.02	<0.01
	Patient (n=30)	0.26	0.10	0.01	
Salivary PH	Control (n=30)	6.71	0.13	0.02	<0.01
	Patient (n=30)	6.59	0.13	0.02	

** N= number*

Salivary Malondialdehyde (MDA) level:
As shown in Table 3, the mean salivary MDA is significantly higher in hypertensive patients compared to the healthy individuals (P<0.01). Mean salivary MDA of the study group was (0.62 µmol/m ±0.17 SD), while that of control group was (0.23 µmol/m ±0.06 SD) **Table 3.**

Table 3: Salivary Malondialdehyde level in hypertensive patients & control

Salivary Malondialdehyde (µmol/m)	N	Mean	±SD	Std. Error	P-value
Control	30	0.23	0.06	0.012	<0.01
Capoten patients	30	0.62	0.17	0.03	

**N= number*

Salivary Superoxide Dismutase (SOD) level:
As shown in **Table 4**, the mean salivary SOD is significantly lower in hypertensive patients compared to the healthy individuals (P<0.01). Mean SOD in the study group (hypertensive patients) was (0.58 µg/ml ±0.21 SD), while that of control group was (1.14 µg/ml ±0.07 SD).

Table 4: Salivary Superoxide Dismutase level in hypertensive patients & control

Salivary Superoxide Dismutase (µg/ml)	N	Mean	±SD	Std. Error	P-Value
Control	30	1.14	0.07	0.01	<0.01
Capoten patients	30	0.58	0.21	0.03	

**N= number*
In this study, a significant negative correlation was found between age of patients and saliva F/R (R= -0.61, P<0.01), **Figure 1.**

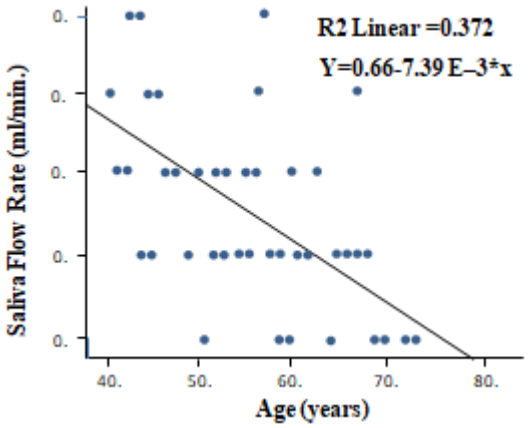

Figure 1: Negative Correlation between Age and Saliva F/R in Hypertensive Patients

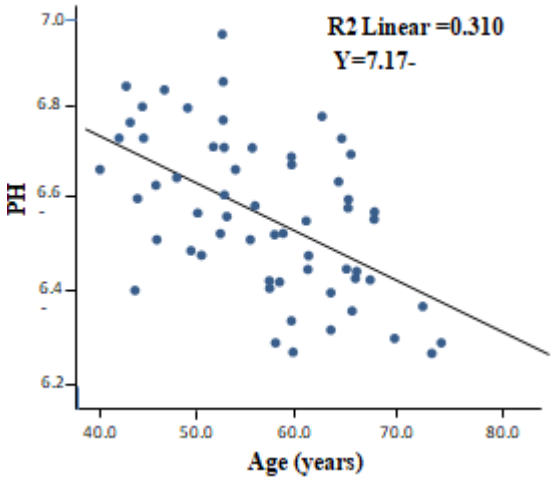

Figure 2: Negative Correlation between Age and Salivary PH in Hypertensive Patients

Also, a negative significant correlation was found between age of patients and salivary PH (R= -0.556, P<0.01), **Figure 2.**

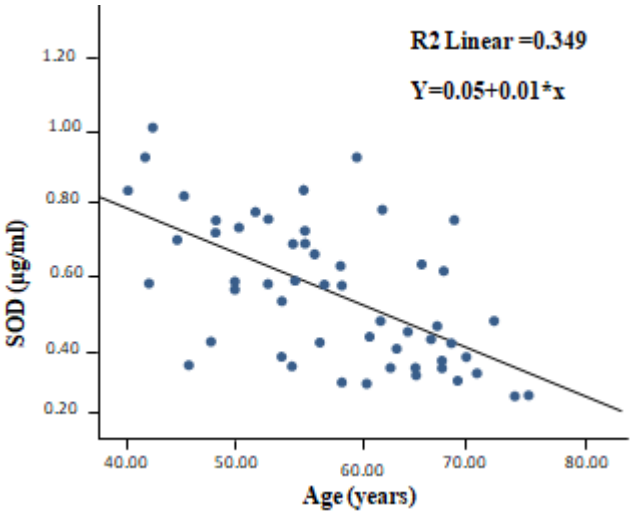

Figure 3: Positive Correlation between Age and MDA of Hypertensive Patients

Considering salivary markers, a significant positive correlation was found between age of patients and salivary MDA level in hypertensive patients (R= 0.591, P<0.01), **Figure 3.**

While age of patients showed a significant negative correlation with salivary SOD (R= -0.570. P<0.01), **Figure 4.**

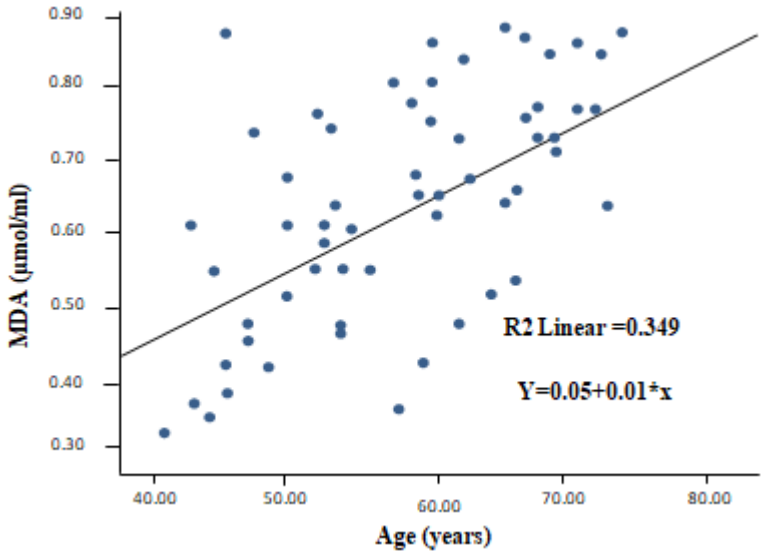

Figure 4: Negative Correlation between Age and SOD of Hypertensive Patients

History of Hypertension and Study Parameters:
The range was (2-12 years) and the mean was (6.3 years). There was a significant positive correlation between MDA and history of hypertension (r=0.704, P<0.01). But, a significant negative correlation was found between history of hypertension and SOD (r=0.658, P<0.01), salivary F/R (r=0.530, P<0.01) and PH (r=0.567, P=0.01), **Table 6.**

Table 6: Correlation between History of Hypertension and Salivary Parameters.

	Variables	Pearson's correlation (r)	P-value
Capoten patient	MDA	0.66	<0.01
	SOD	-0.60	<0.01
	F/R ml/min.	-0.56	<0.01
	PH	-0.35	0.05

4. Discussion

Hypertension is a serious medical problem occurs when blood flows with force greater than normal. Capoten is an angiotensin converting enzyme (ACE) inhibitor. This enzyme responsible for the conversion of angiotensin I to angiotensin II.[6] In this study, 30 patients who were previously diagnosed with hypertension were enrolled. Those patients were under either Capoten (1-8) years. In this study, generally the age of hypertensive patients showed no statically significant difference from normotensive individuals (55.10, 54.77 year). However, hypertensive patients were older than normotensive control subject, which comes with the fact that hypertension is systemic disease mostly of old age. [16]

4.1 Salivary Flow Rate and PH

In the present study, salivary F/R of hypertensive patients was significantly lower than normal control subjects (0.26, 0.37 ml/min). These findings are in agreement with **Böhm, et al., (1985)** [18] who found that salivary F/R is lower in borderline hypertensives than in normotensives. The data support the assumption that in subjects with hypertension the parasympathetic influence on the salivary glands is reduced. This could be due to the effects of medications (Capoten).[18] Also Capoten prevents the conversion of angiotensin I to angiotensin II by inhibition of ACE, [19] resulting in decreased plasma angiotensin II. The reduction of angiotensin II leads to decreased aldosterone secretion, and as a result, small increases in serum potassium may result along with sodium and fluid loss, [18] and this in turn could lead to decrease salivary F/R. Also as the salivary F/R decrease, the concentrations of total protein, sodium, calcium, chloride, and bicarbonate as well as the PH decrease to various levels, whereas the concentrations of inorganic phosphate and magnesium raised.[20]

This disagree with **Nimma, et al. (2016)** [21] who found that there was no significant relation between hypertension and unstimulated salivary F/R. Also,**kagawa, et al., (2013)** [22] found no significant correlation between either hypertension or intake of antihypertensive medication and unstimulated salivary F/R, which also disagree with the current study.

Considering salivary PH, hypertensive patients showed significantly lower PH than normal control subjects (6.59, 6.71). This finding is similar to **kagawa, et al., (2013)** [22] who found a significant correlation between either hypertension or intake of antihypertensive medication and pH of unstimulated saliva. Several studies have been reported that the reduction in salivary flow rate is also the cause of reduction in salivary PH/salivary buffering capacity in individuals, [23] and the electrolytic concentration and tonicity of saliva decrease with decreasing salivary flow rates. [24]

4.2 Salivary markers

4.2.1 Salivary Malondialdehyde level

Malondialdehyde is one of the most reliable markers that determine oxidative stress in clinical situations.(13) The mean salivary malondialdehyde was significantly higher in hypertensive patients compared to the apparently healthy individuals. This agree with **Al-Rawi, et al., (2008)**[25]who found that MDA level was significantly higher in hypertensive patients than that in healthy individual, also**Ahmad et al., (2013)**[26] found that the MDA levels was significantly increased in the hypertension groups as compared to those of the control group, also**Nwanjo et al.,**

(2007)[27] and**Mahdi et al., (2002)**[28] demonstrated an increase in the MDA levels in the essential hypertension cases. which can be attributed to ROS contributes to the etiology of hypertension in humans.[15] Superoxide anion is produced by stimulation of the angiotensin II/angiotensin II type I receptor and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase by angiotensin II which in turn contribute in oxidation process products.[29]

In human with hypertension, ROS may increase due to a diminution of the activity of antioxidant enzymes, [30] which could lead to increase oxidation process and in turn increasing lipid peroxidation process and its products (MDA).

4.2.2 Salivary Superoxide Dismutase level

The mean salivary SOD was significantly lower in hypertensive patients compared to the apparently healthy individuals. This agree with **Ahmad, et al., (2013)**[26] who found that the activities of SOD was significantly lower in the hypertensive patients as compared to those in the normotensive subjects. The data support the assumption that in subjects with hypertension have impaired endogenous and exogenous antioxidant defense mechanisms, [15] also hypertensive patients have reduced activity and decreased content of antioxidant enzymes, including super oxide dismutase, glutathione peroxidase, and catalase. [31]

This disagree with**Al-Rawi, et al., (2008)** [25]who found that SOD level significantly higher in hypertensive patient than that of normotensive one.

Considering the correlation between age and salivary F/R, a significant negative correlation was found between age of patients and salivary F/R and this agreed with**Heintze, et al., (1983)**[32]**; Pendersen, et al., (1985)**[33]**; Cowman, et al., (1994)**[34]**; Michael, (1998)**[35]who found that a reduction in salivary F/R with aging, but disagreed with **Heft., et al., (1984)**[36] who found that there is no significant correlation between age and salivary F/R. This could be due to the effect of antihypertensive medications. [18]

Also, could be attributed to The effect of aging process on physiological homeostasis which can be separated in to two different major pathways, Primary and secondary aging as proposed by **Busse, (1997).**[37]

According to**Narhi, et al., (1992),**[38]the concept of primary aging (chronological) is an alteration in physiological function with advancing age and is independent of extrinsic of physical and psychological disturbances such as stress, trauma and disease. While, secondary aging implies the result of external influences including systemic diseases and therapeutic treatment. It's well-recognized that alteration of salivary function in the elderly are commonly associated with age related diseases (secondary aging).

Since the reduction in salivary F/R could be a cause of reduction in Salivary PH/salivary buffering capacity in individuals. [25] So, a negative significant correlation was found between age of patients and salivary PH.

A significant positive correlation was found between age of patients and salivary MDA level in hypertensive patients. While age of patients showed a significant negative correlation with salivary SOD. These findings suggested that increased lipid peroxidation in patients may be caused by increased free radical production and/or decreased antioxidant defense, which agree with the previous studies**GoncaAkbulut, et al., (1999)**[39]**; Mine ErdenİInal, et al., (2001)**[40]**; RamazanOzcankaya, et al., (2002)**[41]**; ÜmitMutluTürkoğlua, et al., (2003)**[42]which hypothesizedthat Increased oxidative stress may play an important role in the aging process or versa verse.

Furthermore,**Ramazan et al., (2002)** [41]concluded that increased malondialdehyde level is a result of aging so that lipid peroxidation increases due to aging.

While,**Gonca, et al., (1999)** [39]suggested that increased levels of lipid peroxidation products might play a role in aging.**Mine ErdenİInal et al., (2001)** [40] **and Ümit, et al., (2003),** [42] hypothesized that higher level of OS plays an important role in the aging process of individual.

A significant positive correlation was found between history of hypertension and salivary MDA level in hypertensive patients suggests a relation between increased oxidative stress and hypertension which is in agreement with **Am et al., (2007)** [43]who reported an association between increased oxidative stress and higher blood pressure.

a significant negative correlation was found between history of hypertension and SOD in hypertensive patients, this could be attributed to the fact that hypertensive patients have reduced activity and decreased content of antioxidant enzymes, including super oxide dismutase, glutathione peroxidase, and catalase.[31] Also, The decrease in the antioxidant enzymes could be due to their inactivation as the result of a continuous exposure to hydrogen peroxide, hydrogen peroxynitrite and other free radicals.[44]

Moreover, a significant negative correlation was found between history of hypertension and salivary F/R in hypertensive patients, this could be the effect of aging. [37] Also a significant negative correlation was found between history of hypertension and salivary PH, this could be due to the reduction in salivary F/R which could be a cause of reduction in salivary PH/salivary buffering capacity in individuals. [23]

5. Conclusion

1) Over production of the free-radicals may lead to increase oxidative stress which leads to oxidative damage to the biological molecules, leading to several chronic diseases.
2) Salivary MDA increases in hypertensive patients which represent an increasing in oxidative process.
3) Salivary SOD decreases in hypertensive patients which represent a decreasing in anti-oxidant enzyme system.
4) Salivary F/R & PH are negatively associated with hypertension due to the effect of antihypertensive medications or in hypertension the parasympathetic influence on the salivary glands is reduced.

5) Salivary F/R & PH are positively associated with age due to the effect of aging process.
6) This study recommends anti-oxidant supplements for patients with hypertension along with antihypertension medications.

6. Suggestion

Further research may use the same design of this study with larger sample of hypertensive patients.
1) Further studies are required with other types of anti-hypertension medications.
2) Study the effect of anti-oxidant supplement on salivary MDA & SOD in hypertensive patients on Capoten.

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