Research Paper Medical Science

The Evaluation of Relationship Between the Adiponectin and Laryngopharyngeal Reflux Desease (A Preliminary Report)

*** Hande Ezerarslan, MD**	Otolaryngologist, Ufuk University Medical School, Department of Otorhinolaryngology, Balgat 06520, Ankara, Turkey * Corresponding Author
Sedat Özdemir, MD	Ufuk University Medical School, Department of Biochemistry Balgat 06520, Ankara, Turkey
Güçlü Kaan Beriat, MD	Ass. Prof., Ufuk University Medical School, Department of Otorhinolaryngology, Balgat 06520, Ankara, Turkey
Selda Demirtaş, MD	Prof., Ufuk University Medical School, Department of Biochemistry, Balgat 06520, Ankara, Turkey

ABSTRACT

Aim
The aim of our study is to identify etiopathology of Laryngopharyngeal Reflux (LPR) disease by determining level of serum adiponectin whose relation is shown between inflamation and oxidation in the present studies.
Materials and Methods
32 patients with LPR (mean age is 29.72 ± 7.06)) and 25 healthy volunteers (mean age 28 ± which is 7.86) were included in our study. The blood serum adiponectin levels, biochemical values and obesity parameters, systolic and diastolic blood pressures were measured and compared within two groups.
Results and Analysis
The BMI, WHR, systolic and diastolic blood pressure values and blood serum glucose, HbA1c, blood urea nitrogen, creatinin and triglyserides values were higher in LPR patients (p< 0,05). The serum adiponectin and high-density cholestrole values noted lower in LPR patient group than healty volunterees (p< 0,05).
Discussion
LPR disease can be one of the diseases that is related to lower plasma adiponectin levels. Many diseases that create cardiovascular and metabolic problems, which adiponectin levels are shown to be low , should be researched in LPR patients whom values of reflux symptom index and reflux finding score are high.

KEYWORDS Laryngopharyngeal reflux, adiponektin, body mass ındex, Blood Chemical Analysis, Blood pressure

Introduction
Laryngopharyngeal reflux (LPR) is the severe retrograde flow of contents of stomach without vomitting or gagging that can reach upper esophageal sphincter. Inflamation after contact between acid and pepsin with larynx mucosa causes symptoms and signs. However; etiopathology is still contraversial. LPR have ranged from 18 to 80% [1, 2].

Adiponectin is a physiologically active polypeptide secreted by adipose tissue which is role in the modulation of inflammation and oxidation has begun to increasingly apparent in the recent years [3,4].

The aim of our study is to identify etiopathology of LPR disease by determining level of serum adiponectin whose relation is shown between inflamation and oxidation in the present studies. In addition; in LPR patients blood serum biochemical values and body mass index, waist / hip ratio, systolic and diastolic blood pressures that have been shown correlate with the level of adiponectin were measured and compared with healthy individuals.

Materials and Methods
Patients with complaints of LPR such as hoarseness, chronic or recurrent cough, frequent need for throat clearing, globus pharyngeus who attented to our ENT clinic without any other laryngeal diseases were evaluated. Patients whose Reflux Symptom Index (RSI) 13 and Reflux Finding Score (RBS) greater than 7 (with a total of 32 (75% female, 25% male, mean age is 29.72 ± 7.06)) and 25 healthy volunteers (72% females, 28% males, mean age 28 ± which is 7.86) were included in our study. Patients who had no systemic disease (diabetes mellitus, hypertension, asthma), acute or chronic non-infectious inflammatory disease, and history of continuous drug (theophylline, nitrates, anticholinergics, calcium channel blockers, oral contraceptives) use, were selected. Patients with a doubt of malignancy and hiatal hernia identified with upper gastrointestinal endoscopy were excluded from our study.

Local etic comitee permission was gained and informed consent was taken from all patients. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guidelines on human experimentation (Ufuk University) and with the Helsinki Declaration of 1975, as revised in 2008.

Both Reflux Symptoms Index (RSI) and Reflux Finding Score (RFS) whose validity and reliability is approved were used to diagnose LPR. Patients and healthy volunteers were asked to fulfill the Reflux Symptoms Index (RSI) form of 9 questions about laryngopharyngeal reflux before examination [5]. Laryngeal findings were recorded with Hopkins 90°, 5,8 mm telescope (Storz, German) indirect laryngoscopy.

Recordings were evaluated by using Reflux Finding Score (RFS) with regards to pseudosulcus vocalis, ventriculer obliteration,

erytema, laryngeal edema, vocal cord edema, diffuse laryngeal edema, interarytenoid hypertrophy, granulation and thick endolaryngeal mucus [6]. For standardization indirect laryngoscopy was performed by a unique medical doctor. In addition recordings from indirect laryngoscopy were evaluated by a ENT medical doctor who had no information about groups. Patients with RSI>13 and RFS>7 were diagnosed as LPR disease.

Weight, body mass index (BMI), circumference of waist and hip, systolic and diastolic blood pressure of the participants were measured early in the morning while participants were hungry and after micturation with wearing only underwear by the same medical doctor. Also age, gender and cigarette and alcohol usage were noted.

Body mass indexes (BMI) were calculated by the formula of BMI = Weight (kg) / Height (m2). BMI between 18-25 kg/m2 were accepted as normal. BMI=25-30 kg/m2 were named overweight and BMI>30 kg/m2 were named obese [7]. Sytolic and diastolic blood pressures were measured from both arms twice after at least 5 minutes of rest as accepted by the Joint National Comitee (JNC) criteria. Mean values were recorded. Waist circumference (cm) was measured parellel to middle point of distance between spina ischiadica major and inferior margin of 12th costa. Hip circumference (cm) was measured with plastic tape from the most prominent protusion of glutea.

Venous blood samples were taken from patients after average of 14 hours hunger in the morning at 08:00-09:00 a.m. Biochemical parameters such as fasting plasma glucose, HbA1c, transaminases, gamma glutamyl transferase, total cholesterol, HDL(High-density Lipoprotein) cholesterol, LDL (Low-density Lipoprotein) cholesterol, triglycerides, and CRP levels were analysed in the same day. 5 ml blood was centrifuged and stored at -24oC till study date for adiponectin. Adiponectin was analyzed with AviBion Human Adiponectin (Acrp30) kit using an ELISA method according to literature [8].

Statistical Method

The obtained data were analyzed statistically by PASW 18 program. Kolmogorov-Smirnov normality test was used whether the distribution of the data have been normal. T-test and Mann-Whitney U test were used to compare the data between groups. For grouped data (yes – no) were evaluated by chi-square analysis. Spearman's correlation test was used to determine level of relationship between intra-group and inter-groups.

Results and Analysis

32 patients with laryngopharyngeal reflux (Group 1) (age min: 19, max: 52) and 25 healthy volunteers(Group 2) (age min: 18, max: 50) were included in the study. There was no difference between the distribution of age and gender between the two groups (respectively p = 0,28; p= 0,52). Demographic information, habits, and basic features of the study groups are shown in Table-1.

Comparison of blood plasma values between the two groups are summarized in Table 2. Analysis of blood serum samples between the two groups of patients with a complete blood count, albumin, ALT (alanine aminotransferase), AST (aspartate aminotransferase), total cholesterol and LDL (Low-Density Lipoprotein), a statistically significant difference between the values have not been determined.

Intra-group analysis of adiponektin blood serum levels in the first group with statistically significant difference between values of other parameters are shown in Table-3.

Discussion

We have assessed adiponectin levels, adiposity parameters and blood plasma biochemistry parameters in laryngopharyngeal reflux patients for the first time in literature.

Adipose tissue is a hormonal organ that secretes adipocytokines. These adipocytokines create effects that can be called negative on metabolism. However, adiponectin differs from other cytokines because of its antioxidant and anti-inflammatory effects.

Adiponectin inhibits adhesion molecule (vascular cell adhesion molecule (VCAM-1), E-selectin and intercellular adhesion molecule (ICAM)-1) of surface expression on endothelial cells [9] and stimulates production of the anti-inflammatory cytokine IL-10 from macrophage [10]. Adiponectin also inhibits the production of reactive oxygen types in human neutrophils [11].

Adiponectin receptors, AdipoR1 (muscle), AdipoR2 (liver), have been identified and activation of these reseptors result in inhibition of inflammation and oxidative stress [12, 13].

It is known that there is negative correlation between TNF- α and adiponectin. Adiponectin expression and secretion from adipocytes is significantly decreased with TNF-α in mice and human cell cultures [14]. Additionally, adiponectin strongly inhibits LPS-induced TNF-α gene expression in macrophages [15].

To sum it up, adiponectin gene expression is down regulated reversibly with TNF-α. Adipose-derived cytokine has anti-inflammatory effect on especially endotelial cells and macrophages.

The mechanism of laryngeal injury is uncertain in LPR disease. Receptor-mediated uptake of pepsin may cause damage at an intracellular level where carbonic anhydrase isoenzyme III has also been implicated [16, 17]. Furthermore, Total anti-oxidant capacity, total oxidant level and oxidative stress index are shown elevated in laryngopharyngeal reflux patients [18]. Adinopectin releated anti-inflammatory and anti-oxidant factors and LPR disease can be thought together and therefore that must be kept in mind in future studies. Whether the high adiponectin consumption may be part of the protective response to the underlying inflammation, or those with low adiponectin (or those less able to increase adiponectin) may be more prone to inflammation in LPR disease.

In our study, adiponectin levels were found lower in LPR patients. İmmune cell defence and inflammatory response against acid and pepsin can be lower in LPR patients. This is supported by the fact between negative correlation between adiponectin levels and symptoms and findings' scores because of inflammation caused by acid and pepsin.

Adiponectin is also sempatic nervous system suppressor which has antioxidant and hypotensive effect because of NO [19, 20]. In men, when blood pressure is high, adiponectin is related with risk factors of cardiovascular disease such as obesity and tip II diabetes mellitus [9, 21, 22, 23]. In our study, low adiponectin levels in LPR patients had higher mean values of systolic and diasytolic blood pressures than healthy population as coherent to literature. By this reason, LPR patients should be under control in aspects of cardiological risks.

Recent studies showed plasma adiponectin concentrations have negative correlation between plasma glucose, insulin and triglyceride levels but positive correlation between plasma HDL cholesterol levels [24, 25]. Yamamoto et al also showed negative correlation between plasma adiponectin levels and fasting plasma glucose, insulin, insulin resistance, total and low-density lipoprotein cholesterol, triglycerides and uric acid and positive correlation between high-density lipoprotein (HDL, high-density lipoprotein) cholesterol in 967 Japanese participants with normal weights [26]. Another study that examined the relationship between adiponectin levels and serum lipid concentrations [27], showed positive correlation between plasma adiponectin and serum HDL cholesterol levels but negative correlation to serum triglyceride, atherogenic index, apo B or apo E in a large number of non-diabetic women with dislipidemia.

In our study; positive correlation between plasma adiponectin

level and HDL cholestrol (ρ (rho) value: 0.551, p value = 0); negative correlation between plasma adiponectin level and fasting plasma glucose, HbA1c, triglycerides, sodium and potassium were found.

In our study, BMI was used to determine total body fat; WHR and waist circumference were used for central obesity. Gavrila A et al, showed adiponectin levels and BMI, WHR and waist circumference had negative correlation [28]. They showed that adiponectin and WHR and waist circumference had better correlation than BMI. Thus; central adiposity was more reliable to determine serum adiponectin levels than total fat mass [29]. As waist circumference is increased, adiponectin levels are decreased suggesting that central obesity and adiponectin levels had inverse ratio and as obesity levels increase adiponectin levels decrease in the same manner. Analogously, in this study negative correlation is showed between BMI, waist circumference, WHR and adiponectin levels. However, relevance between adiponectin and BMI is higher than waist circumference and WHR.

In the literature, there is only a few study which examined the relationship between BMI of patients and the occurrence of LPR. Halum et al. reviewed 285 patients with LPR and did not correlate with increasing BMI; however, Saruç et al. showed high BMI values in LPR patients [30, 31].

In this study BMI and WHR were shown higher in the LPR patients than healthy population. Mean value of BMI of LPR patients was 25,5 as named overweight but not obese. Furthermore, as WHR was high in LPR patients it can be thought that central obesity may be a risk factor as well in LPR.

Conclusion

LPR disease can be one of the diseases that is related to lower plasma adiponectin levels. In addition of anti-inflammatory affect of adiponectin, lack of this cytokine can also result in the damage to larynx mucosa. This can also explain the reason that some patients have higher degree of acid damage. Many diseases that create cardiovascular and metabolic problems, which adiponectin levels are shown to be low , should be researched in LPR patients whom values of RSI and RFS are high. In addition, patients who have low adiponectin levels can benefit from anti-oxidant and anti-inflammatory drugs addition to proton pump inhibitors, and life style changes must include either anti-reflux or cardiac and metabolic precautions. Many more researches with more patients and more prolonged time should be made to support this thesis.

Conflict(s) of Interest

None

Table 1: Comparison of the basic features, finding and symptoms of reflux and habits of patients with laryngopharyngeal reflux and patients in the control group (n: Number of case, sd: Standart deviasion, BMI: Body mass index, WHR: Waist to hip ratio, SBP: Sistolic blood pressure (mmHg), DBP: Diastolic blood pressure (mmHg), RSI: Reflux symptom index, RFS: Reflux finding score).

	Group 1			Group 2			p value
	n	mean	sd	n	mean	sd	
Age	32	29,72	7,06	25	28	7,86	0,39
BMI (kg/ m2)	32	25,5	4,71	25	22,57	2,59	0,02
WHR	32	0,86	0,05	25	0,81	0,06	0,02
Smoker (%)	32	18,8		25	28		0,3
Alcohol usage (%)	32	25		25	28		0,52
SBP (mmHg)	32	123,6	8,06	25	115,8	5,14	0
DBP (mmHg)	32	82,97	5,21	25	62,8	6,93	0
RSI	32	24,56	4,44	25	6,28	1,9	0
RFS	32	13	3,11	25	3,69	1,55	0

Table 2: (Adiponectin: Serum adiponectin (ng/dL); Glucose: Fasting blood glucose (mg/dL); BUN: Blood urea nitrogen (mg/dL); Crea: Creatinine (mg/dL); TG: Triglycerides; HDL: High-density Lipoprotein (mg/dL); LDL: Low-density Lipoprotein (mg/dL);TG: triglycerides (mg/dL))

	Group 1			Group 2			p value
	n	mean	sd	n	mean	sd	
Adiponectin	32	105,87	37,6	25	155,03	44,15	0
Glucose	32	88,32	7,69	25	73,95	5,61	0
HbA1c	32	5,18	0,38	25	4,51	0,36	0
Na	32	146,51	2,87	25	144,09	3,98	0,01
K	32	4,68	0,36	25	4,08	0,31	0
BUN	32	15,4	3,44	25	11,56	2,91	0
Crea	32	0,80	0,14	25	0,66	0,12	0,01
HDL	32	46,92	4,09	25	61,61	4,80	0
TG	32	96,23	17,84	25	76,71	10,46	0

Table 3: The statistically significant relationship between adiponectin levels and the other parameters in group 1(ρ: Rho value; p: p value; Wc: Waist circumference, Hc: Hip circumference, BM: Body mass; BMI: Body mass index, Alb: Albumin).

		Wc	Hc	BM	BMI	HbA1c	Glucose	Na	Alb
Adiponectin	ρ	- 0,427	- 0,443	- 0,429	- 0,483	- 0,60	- 0,707	- 0,373	0,546
	p	0,015	0,011	0,014	0,005	0	0	0,036	0,001

REFERENCES

1. Gatta L et al Meta-analysis: the eYcacy of proton pump inhibitors for laryngeal symptoms attributed to gastro-oesophageal reXux disease. Aliment Pharmacol Ther 2007;25(4):385–392 . | 2. Koufman JA The otolaryngologic manifestations of gastroesophageal reXux disease (GERD): a clinical investigation of 225 patients using ambulatory 24-hour pH monitoring and an experimental investigation of the role of acid and pepsin in the development of laryngeal injury. Laryngoscope 1991; 101(4 Pt 2 Suppl 53):1–78. | 3. Frühbeck G, J Gomez-Ambrosi, FJ Muruzabal, MA Burrell. The adipocyte : a model for integration of endocrine and metabolic signalling in energy metabolism regulation. Am J Physical Endocrine Metal 2001. 280 : E827-E847. | 4. Fruebis J, Tsao TS, Javorsehi S, Ebbets-Reed D, Erickson MR, Yen FT et al. Proteolitic eleavage product of 30-kDa adipocyte;complement related protein increases fatty acid oxidation inmuscle and causes weight loss in mice. PNA.s 2001;98:2005-2010. | 5. Belafsky PC, Postma GN, Koufman JA. Validity and reliability of the reflux symptom index (RSI). J Voice 2002;16:274-7. | 6. Belafsky PC, Postma GN, Koufman JA. The validity and reliability of the reflux finding score (RFS). Laryngoscope 2001;111:1313-7. | 7. Deurenberg P,Yap m.The assesment of obesity:methods for measuring body fat and global prevalance of obesity.Baillieres best Pract Res Clin Endocrinol Metab 1999 A;13(1):1-11 | 8. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein , adiponectin. J Clin Endocrinol Metab. 2001 Aug, 86 (8) :3815-9. | 9. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y: Novel modulator for endothelial adhesion molecules : adipocyte-derived plasma protein adiponectin. Circulation 1999, 100:2473-2476. | 10. Kumada M, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K, Maeda K, Nagaretani H, Kishida K, Maeda N, Nagasawa A, Funahashi T, Matsuzawa Y: Adiponectin specifi cally increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. Circulation 2004, 109:2046-2049. | 11. Magalang UJ, Rajappan R, Hunter MG, Kutala VK, Kuppusamy P, Wewers MD, Marsh CB, Parinandi NL: Adiponectin inhibits superoxide generation by human neutrophils. Antioxid Redox Signal 2006, 8:2179-2186. | 12. Yoon MJ, Lee GY, Chung J-J, Ahn YH, Hong SH, Kim JB: Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase, p38 mitogen-activated protein kinase, and peroxisome proliferator-activated receptor alpha. Diabetes 2006,55:2562-2570. | 13. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T: Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002, 8:1288-1295. | 14. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y: PPAR gamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes 2001, 50:2094-2099. | 15. Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y: Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood 2000, 96:1723-1732. | 16. Gill GA, Johnston N, Buda A, Pignatelli M, Pearson J, Dettmar PWet al. Laryngeal epithelial defenses against laryngopharyn-geal reflux: investigations of E-cadherin, carbonic anhydrase isoenzyme III, and pepsin. Ann Otol Rhinol Laryngol2005; 114:913–21. | 17. Johnston N, Knight J, Dettmar PW, Lively MO, Koufman J. Pepsin and carbonic anhydrase isoenzyme III as diagnostic markers for laryngopharyngeal reflux disease. Laryngoscope 2004;114:2129–34. | 18. Mutlu Ö, Kar M, Yıldız Zeyrek F, Aksoy N, Taşkınn A. Total antioxidant capacity, total oxidant level and oxidative stress index in patients with laryngopharyngeal reflux with Helicobacter pylori IG G positivity. J Med Updates 2013;3(1):25-30. | 19. Omae T, Nagaoka T, Tanano I, Yoshida A. Adiponectin-Induced Dilation of Isolated Porcine Retinal Arterioles via Production of Nitric Oxide From Endothelial Cells. Invest Ophthalmol Vis Sci. 2013 Jul 10;54(7):4586-94. | 20. Karimi N, Roshan VD. Change in adiponectin and oxidative stress after modifiable lifestyle interventions in breast cancer cases. Asian Pac J Cancer Prev. 2013;14(5):2845-50. | 21. Arita Y. Kihara S. Ouchi N. Takahashi M. Macda K. Miyagawa J et al. Paradoxical decrease of an adipocyte specific protein,Adiponectin in obesity.Biochemical and Biophysical Research Comunications 1999;257:79- 83 | 22. Yamamoto Y, Hirose H, Miyashita K, Nishikai K, Saito L Taniyama M et al. PPARgamma2 gene Pro12Ala polymorphism.may influence serum level of an adipocyte-derived protein, adiponectin in the japanese population. Metabolism1 2002;51:1407-1409. | 23. Stefan N, Vozarova B, Funahashi T, Matsuzawa Y,Weyer C,Lindsay RS et al. Plasma adiponectin concentrations associatedwith skeletal muscle insulin receptor tyrosine phosphorlation.and low plasma concentration precedes a decrease in whole bodyinsulin sensitivity in humans. Diabetes 2002;51:1884-1888. | 24. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y et al. Plasma concentrations of a novel adipose-specific protein, adiponectin, in type 2 diabetic patients. Arteriosclerosisand Thrombosis vaskuler Biology 2000;20:1595-1599. | 25. Stejskal D, Ruzicka V, Adamovska S, Jurakova R, Proskova J, Jedelsky L, et al. Adiponectin concentrations as a criterion of metabolic control in persons with type 2 diabetes mellitus? Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2003; 147: 167-72. | 26. Yamamoto Y, Hrose H, Saito i, Tomita M, Taniyama M, Matsubara Ket al. Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high density lipoprotcin-cholesterol independent of body mass index, in the Japanese population. Clinical Science 2002 103 137-142. | 27. Kondo H. Shimomura I,Matsukawa y, Kumada M, Takahashi M,Matsuda M et al. Association of adiponectin mutation with type 2 diabetes. A candidate gene for the insulin resistance syndrome. Diabetes 2002 51 2325-2328. | 28. Gavrila A, Chan JL, Yiannakouris N, Kontogianni M, Miller LC, Orlova C, Mantzoros CS. Serum adiponectin levels are inversely associated with overall and central fat distribution but are not directly regulated by acute fasting or leptin administration in humans: cross-sectional and interventional studies. J Clin Endocrinol Metab. 2003 Oct;88(10):4823-31. | 29. Moayyedi P: Barrett's esophagus and obesity: the missing part of the puzzle. Am J Gastroenterol 2008; 103:301-303. | 30. Halum SL, Postma GN, Johnston C, Belafsky PC, Koufman JA. Patients with isolated laryngopharyngeal reflux are not obese. Laryngoscope. 2005 Jun;115(6):1042-5. | 31. Saruç M, Aksoy EA, Vardereli E, Karaaslan M, Ciçek B, Ince U, Oz F, Tözün N. Risk factors for laryngopharyngeal reflux. Eur Arch Otorhinolaryngol. 2012 Apr;269(4):1189-94. |