Cotinine Levels Influence the Risk of Rupture of Brain Aneurysms

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Abstract

The relationship between nicotine levels in smokers and brain aneurysm has never been determined. To assess the levels of cotinine in smoker patients with ruptured and unruptured brain aneurysm and the risk of aneurysm rupture we quantified cotinine levels in smoker patients with ruptured or unruptured brain aneurysms. We identified a control group of smokers and nonsmokers without brain aneurysm. Out of 182 participants cerebral aneurysms were statistically significantly associated with smoking (P<0.001) and female sex (P=0.006). Cotinine levels were significantly correlated with both the presence (P=0.009) and the rupture (P=0.002) of brain aneurysms. Compared with nonsmokers, smokers had a 5-fold higher risk of having a brain aneurysm (OR, 5.72; 95% CI, 2.96–11.07; P<0.001). The risk of rupture of brain aneurysms increased by 50% with each cotinine unit and was 4-fold higher with cotinine levels between 4 and 6 (OR, 3.75; 95% CI, 1.48–9.53; P=0.005). With increasing age, the cotinine level decreased (P <0.001, rho= −0.28), declining by 2% with each year of age. In the whole population, the probability of a ruptured aneurysm in patients with cotinine levels between 4 and 6 was higher than in those with levels between 0 and 3 (OR, 5.55; 95% CI, 1.08–28.5; P=0.040). Our results suggest that high cotinine levels in smokers with brain aneurysm, rather than size, are significantly associated with high rupture risk. Cotinine levels decrease with age, possibly reducing the risk of formation and rupture of a brain aneurysm.

1. Introduction

Smokers have a total life expectancy at least 10 years shorter than never-smokers or approximately 4 years younger [19, 38, 48]. Smoking is an independent and the most important risk factor for intracranial aneurysm rupture [12, 20, 47]. The prevalence of smokers among patients with ruptured aneurysm exceeds 70% [21, 28], but if former smokers are included it reaches almost 80% (Table 1) [29, 35, 54]. Similar prevalence is reported in those with unruptured intracranial aneurysm, and the majority of studies confirm a female to male ratio of 2–3:1 [7, 15, 17, 25, 29, 31, 35, 37, 50, 54]. Nicotine is one of the many and more harmful chemicals in tobacco, but there are no studies evaluating the level of nicotine in smokers with intracranial aneurysm. Tobacco smokers consume approximately 1 mg of nicotine with each cigarette [4, 41, 44]. Nicotine increases the aneurysm rupture rate in mice by acting on the α7*-nAChR of vascular smooth muscle cells [24].

Table 1

Prevalence reported in the literature of smokers and nonsmokers among patients with brain aneurysm. Smokers and former smokers are well represented in both the ruptured and unruptured aneurysm groups, exceeding the Koskinen (77%), Woo (79%), Juvela (71%), Korja (71%), Muller (75%) or almost reaching more than 2/3 of the total reported. In all series, female patients outnumbered male patients. S: smoker; ex-S: former smoker; nS: nonsmoker

	n° of patients	ruptured aneurysms			unruptured aneurysms			Female	male
		S	**ex-S**	**nS**	**S**	**ex-S**	**nS**		
Okamoto K. (2005)	201	**201**			-			**124**	77
		86	-	115	-	-	-	**(62%)**	(38%)
		(43%)		(57%)					
Koskinen L-OD (2006)	99	**99**			-			**79**	41
	(120)	**55**	**21**	23	-	-	-	**(66%)**	(34%)
		(55%)	**(22%)**	(23%)					
Woo D. (2009)	335	**335**			-			**229**	106
	(339)	**210**	**53**	72	-	-	-	**(68%)**	(32%)
		(63%)	**(16%)**	(22%)					
Juvela S. (2013)	123	**28**			**95**			**76**	66
	(142)	**20**	0	8	**38**	**28**	29	**(53%)**	(47%)
		(71%)	(0%)	(29%)	**(40%)**	**(30%)**	(31%)		
Vlak M. (2013)	456	**250**			**206**			**326**	130
		155	-	95	96	-	110	**(71%)**	(29%)
		(62%)		(38%)	(47%)		(53%)		
Korja M. (2014)	82	**28**			**54**			**61**	57
	(118)	**20**	-	8	32	-	22	**(52%)**	(48%)
		(71%)		(29%)	(59%)		(41%)		
Kang H. (2015)	519	**178**			**341**			**323**	196
		62	-	116	87	-	254	**(62%)**	(38%)
		(35%)		(65%)	(26%)		(75%)		
Ho A. (2015)	199	**117**			**82**			**154**	45
		61	-	56	36	-	46	**(77%)**	(33%)
		(52%)		(48%)	(44%)		(56%)		

Lindbohm J. (2016)	491	**491**			-			**270**	221
		213 (43%)	70 (14%)	208 (42%)	-	-	-	**(55%)**	(45%)
Can A. (2017)	4.701	1.302			**3.399**			**3.366**	1.035
		525 (40%)	310 (24%)	467 (36%)	872 (26%)	839 (25%)	1.688 (50%)	**(72%)**	(28%)
Feng X. (2018)	381	**127**			**254**			**222**	159
		43 (34%)	7 (6%)	77 (61%)	51 (20%)	17 (7%)	186 (73%)	**(58%)**	(42%)
Figueredo (2019)	189	**72**			**117**			**138**	51
		25 (35%)	-	47 (65%)	28 (24%)	-	89 (76%)	**(73%)**	(27%)
Müller (2019)	203	**111**			**92**			**144**	56
		67 (60%)	**17 (15%)**	27 (25%)	**56 (61%)**	**(21%)**	17 (18%)	**(71%)**	(29%)

The variable number and quality (light, mild, or regular) of daily smoked cigarettes, as well the pH of the smoke (pH 8 allows a ~2.5-fold higher nicotine concentration than pH 7, and 4-fold higher than pH 6) and the smoking alternatives (electronic cigarette, cigar, pipe, plain tobacco, hookah, oral tobacco) make it difficult to determine exactly how much nicotine is consumed by each smoker [2]. Nicotine is metabolized in the liver to cotinine, and determination of the cotinine level, the major metabolite of nicotine and index of exposure to tobacco smoke, can be performed to ascertain how much nicotine is absorbed. In the present study, we examine the cotinine level in smoker patients with intracranial aneurysm, aiming to better demonstrate the relationship between nicotine absorption level and the risk of formation and rupture of an intracranial aneurysm.

2. Materials And Methods

According to the Helsinki principles and previous ethics committee approval (CE 5224, January 29 2019), a single-center, cross-sectional observational, case-control study was conducted in patients with intracranial ruptured or unruptured aneurysm with regard to their smoker or nonsmoker status [55]. Patients were consecutively recruited from the Emergency Department and the Neurosurgery ward within 8 hours of arrival, or from the outpatient clinic (Group A). At the same time, a randomly selected control group of individuals submitted to neuroradiological evaluations for chronic headache or other neurological symptoms but without intracranial aneurysm was collected (Group B). Smokers and former

smokers were considered together in both groups, since a brain aneurysm could develop even many years before smoking cessation. Based on their size, intracranial aneurysms were classified as small (<7 mm), medium (7–12 mm), large (13–25 mm), and giant (>25 mm). Patients with two or more aneurysms were considered to have multiple aneurysms. Cotinine levels were assessed in salivary or urinary samples, according to the degree of consciousness and understanding of each participant. A fast test strip that gives semi-quantitative results (levels 0 to 6: 0 to >1000 ng/mL) was employed (NicAlert, Nymox Pharmaceutical Corporation, St. Laurent, QC, Canada). Inclusion criteria for both groups were age ≥25 and neuroradiological imaging (CT, MRI, or angiography) demonstrating or excluding an intracranial aneurysm. Exclusion criteria were presence of neurodegenerative diseases, cerebral hemorrhage in the absence of aneurysm, and pregnancy. Informed consent to analyze their personal data was obtained from all subjects or their next of kin prior to their inclusion in the study.

Statistical analysis

In cases, cotinine levels (in smokers and nonsmokers) were related to patient age and sex, the size and number of aneurysms, and the site and state of the aneurysm (ruptured or unruptured). In controls, cotinine levels (in smokers and nonsmokers) were related to age and sex. A t-test was used to evaluate the difference in age between the two groups. The chi-squared test was used to evaluate the relationship between two categorical variables. The Mann-Whitney test was used to assess the cotinine distribution between the two groups. Spearman's correlation was used to evaluate the correlation between two variables. Logistic regression with OR calculation and 95% confidence interval was used to assess the probability of the risk of rupture of one group compared with another. A Poisson regression was applied to evaluate how much cotinine decreases for each year of age. Statistical analyses were performed using Stata software 16.1. A *P*-value <0.05 was considered statistically significant.

Data availability

The dataset analyzed in this study is not publicly available because of restricted access but informations about the dataset is available from the corresponding author on reasonable request.

3. Results

Due to the COVID-19 pandemic, the study was halted at the half-way point because the regional government requested the university hospital to modify the number of available intensive care and clinical beds, diverting patients with neurosurgical pathologies to other non COVID-19 hospitals. Before this halt, 182 cotinine levels were collected from 182 participants. Patients with aneurysm were included in group A, and a control group (B) was used as a comparison.

Group A

Group A included 86 patients with ruptured or unruptured aneurysms, of whom 61 were women and 25 were men (F to M ratio 2.4:1) (Table 2). The average age was 60.31 years (28–84 years), and there were 34 patients (39.5%) with ruptured aneurysms and 52 patients (60.5%) with unruptured aneurysms. Sixty-one patients (71%) had a single aneurysm and 25 patients (29%) had multiple aneurysms, for a total of 123 aneurysms. The average age of patients with ruptured aneurysms was 57 years, and in patients with unruptured aneurysms it was 62 years. Women predominated in both the ruptured aneurysm group and the unruptured aneurysm group: 21 women vs 13 men, and 40 women vs 12 men, respectively. The size of the aneurysms was between 1 and 26 mm: 46 aneurysms were small (37.4%), 60 were medium-sized (48.8%), 16 were large (13%), and one was giant (0.8%). The average size of the ruptured aneurysms was 6.94 mm, and that of the unruptured aneurysms was 7.04 mm (P=0.468). The most common aneurysm location was the internal carotid artery (54 aneurysms, 44%), followed by the middle cerebral artery (32 aneurysms, 26%), the anterior circulation (27 aneurysms, 22%), and the posterior circulation (10 aneurysms, 8%). There were 17 ruptured aneurysms located in the anterior circulation (50%), 7 in the middle cerebral circulation (20.6%), 6 in the internal carotid circulation (17.6%), and 4 in the posterior circulation (11.8 %). The prevalence of brain aneurysm in smokers compared with nonsmokers was 67.5% versus 32.5% (58 smokers and 28 non smokers respectively: P<0.001), and 24 (41.4%) of these had a ruptured aneurysm. More than 70% of ruptured aneurysms (24/34) were in smoker patients. There was no statistically significant difference between smoking and multiple aneurysm (P=0.418).

Table 2
Clinical data in our series of patients with aneurysms

Aneurysm	No. (%)	Mean	Female/Male	*P*
Women	61 (71)	-	-	
Men	25 (29)	-	-	
Average age (years)	60.31 (28-84)	-	-	
Single	61 (70.93)	40 smokers (65.5%)	-	0.418
Multiple	25 (29.07)	18 smokers (72%)	-	
Size	46 small	37.4%	-	
	60 medium	48.8%		
	16 large	13%		
	1 giant	0.8%		
Location	54 ICA	44%	-	
	32 MCA	26%		
	27 AC	22%		
	10 PC	8%		
Ruptured	34 (39.5%)	57 years	21/13	
Unruptured	52 (60.5%)	62 years	40/12	
Smokers	58/86 (67.4%)	24 ruptured aneurysm (41.4%)	-	<0.001
Nonsmokers	28 (32.5%)	10 ruptured aneurysm (35.7%)	-	
Ruptured smoker	24/34 (70.6%)			
Unruptured smoker	10/34 (29.4%)			

The most common cotinine level was 1 (40 patients, 46.5%), followed by levels 5 (13 patients, (11.1%), 4 (12 patients, 14%), 3 (8 patients, 9.3%), 6 (5 patients, 5.8%), 0 (5 patients, 5.8%), and 2 (3 patients, 3.5%) (Table 3).

Table 3
Cotinine levels and aneurysm

Cotinine level	No. patients	%	Ruptured	%	Unruptured	%	*P*
0	5	5.8	0	-	5	9.6	
1	40	46.5	13	38.2	27	51.9	
2	3	3.4	1	2.9	2	3.8	
3	8	9.3	2	5.8	6	11.5	
4	12	13.9	5	14.7	7	13.4	0.002
5	13	15.1	9	26.4	4	7.6	
6	5	5.8	4	11.7	1	1.9	
Total	*86*		*34*		*52*		

In smokers with aneurysm, the median cotinine level was 4 (interquartile range 1–5), while in non-smoking cases it was 1 (interquartile range 1–1) (P<0.021).

Smoker patients with high cotinine levels (≥4) had a significantly higher percentage of ruptured (18 patients, 52.8%) vs unruptured aneurysms (12 patients, 23%) (P=0.002) (Fig. 1). In patients with a cotinine value between 4 and 6, the probability of rupture of a cerebral aneurysm was 4-fold higher than in those with a cotinine value between 0 and 3 (OR=3.75, P=0.005). On average, with each step up in cotinine level the risk of rupture increased by about 50%.

Group B

The control group included 96 individuals without aneurysm, of which 49 were women and 47 were men (F to M ratio 1:0.96). The average age was 61.7 years (31–81 years). Among the controls, 27 (28%) were smokers and in these the median cotinine level was 4 (interquartile range 4–5), and in non-smoking controls it was 1 (interquartile range 1–1). The most represented cotinine level was 1 (61 patients, 63.54%), followed by levels 5 (12 patients, 12.5%), 4 (9 patients, 9.4%), 0 (9 patients, 9.4%), 3 (3 patients, 3.1%), 2 (1 patient, 1.03%), and 6 (1 patient, 1.03%).

Comparison between groups A and B

The distribution of smokers and nonsmokers in the two groups is evidence that smoking is significantly associated with the presence of a brain aneurysm. The percentage of smokers with aneurysm was higher than it was in the controls without aneurysm (67.4% vs 28.1%, P<0.001). The likelihood of developing an aneurysm was 5-fold higher in smokers than in nonsmokers (P<0.001). When calculating the odds ratio in both the univariate analysis and the multivariate analysis, corrected for smoking status, the risk of having a brain aneurysm was 5-fold higher in smokers compared with nonsmokers (OR, 5.72; 95% CI, 2.96–

11.07; *P*<0.001). Among smokers, the likelihood of having an aneurysm was 3-fold higher in women than in men (OR, 2.69; 95% CI, 1.36–5.30; *P*=0.004).

The prevalence of women (70.9% vs 51%) was higher in cases than in controls (*P*=0.006). Considering groups A and B together (182 smokers and nonsmokers), cotinine level 1 was the most represented (101 subjects, 55.5%, of which 97 were nonsmokers), followed by levels 5 (25 subjects, 13.7%), 4 (21 subjects, 11.6%), 3 (11 subjects, 6%), 6 (6 subjects, 3.3%), 0 (14 subjects, 7.7%), and 2 (4 subjects, 2.2%). There was a significant difference (*P*=0.009) in the distribution of cotinine between cases and controls (Fig. 2). Although the median value was 1 for both groups, the percentage of cases in the three highest levels (cotinine levels 4, 5, and 6) was 35%, while in the controls it was 23%.

In relation to age, the cotinine level in all study participants showed a negative correlation (decreasing with increasing age), and the Kruskal-Wallis test indicated that there was a significant difference between the groups (*P*=0.009). This was confirmed in nonsmokers and smokers (*P*<0.001, rho= −0.28). Poisson regression shows that the incidence rate ratio was 0.98 (95% CI, 0.98–0.99; *P*<0.001). The interpretation of the incidence rate ratio is similar to that of the OR and in this case it indicates that there is a 2% decline in cotinine with each year of age. Figure 3 shows the distribution of cotinine levels with respect to age.

Paired samples

Analysis of 36 patients paired for age, sex, and smoker status revealed that in smokers, cotinine levels 4, 5, and 6 were more represented in patients with ruptured aneurysm (56.2%) than in patients with unruptured aneurysm (15%). The median cotinine level was higher in patients with ruptured aneurysms than it was in those who did not have a ruptured aneurysm (*P*=0.001). The probability of having a rupture increased as the cotinine level increased (OR, 1.58; 95% CI, 1.04–2.39; *P*=0.032) and for each unit increase in cotinine level the risk of rupture increased 1.6-fold. There was a significant negative correlation between cotinine and age (cotinine declined with increasing age) in the whole sample (rho= −0.38, *P*<0.001), the controls (rho= −0.43, *P*=0.010), and the cases (rho= −0.37, *P*=0.026). To evaluate how much cotinine decreases with every year of age (using a Poisson regression that also considered the pairing of the subjects), the incidence rate ratio was 0.97 (95% CI, 0.95–0.99; *P*<0.001). There was a 3% reduction in cotinine with every year of age (according to Poisson regression). Considering all the aneurysms, subjects with cotinine levels of 4–6 had a greater probability of having a rupture than those with levels of 0–3 (OR, 5.55; 95% CI, 1.08–28.5; *P*=0.040).

Negative Results

No significant relationship between the level of cotinine and the size of the aneurysm was found, considering the entire group (*P*<0.115), or only smokers (*P*=0.269), former smokers (*P*=0.67), or nonsmokers (*P*=0.992).

4. Discussion

The previous literature showing the strong relationship between smoking and brain aneurysm, and female preponderance, was confirmed (Table 1). Smoking is a dangerous addiction that causes vascular damage in the brain and is significantly related to abdominal and thoracic aortic, iliac, and renal aneurysms [6, 8, 10, 26, 40, 53].

Addressing smoking and brain aneurysm from a different perspective, this study revealed new information. The approximate number of cigarettes consumed, packs per day, or years of smoking are inaccurate to determine the real nicotine absorption by the smoker, but measuring the level of salivary or urinary cotinine can overcome this uncertainty. Our first findings are that smokers have a 5-fold higher brain aneurysm formation risk compared with nonsmokers and that, rather than size, individuals with high cotinine levels (200–1000 ng/mL) have a 4-fold rupture risk compared with those with lower cotinine levels. These results confirm that smoking cessation or extreme reduction is mandatory advice for patients with diagnosed unruptured brain aneurysms [3, 5, 9, 16, 22, 23, 27, 33, 39, 54]. Nevertheless, the risk of rupture of an already formed aneurysm persists even after quitting smoking [7, 42, 45]. This is the reason we included former smokers with ruptured or unruptured brain aneurysms in the smoker category, as an aneurysm may have formed many years before smoking cessation. In line with the previous literature indicating smoke consumption as a factor in the formation and rupture of brain aneurysms, our results show that for each unit increase of cotinine the risk of rupture increases by 50%, and that there is a 4-fold increase in rupture risk in patients with cotinine levels ≥4.

It can thus be cautiously stated that if a smoker manages to stop or drastically reduce the number of cigarettes smoked and consequently achieves a cotinine level of 1 (which could presumably be done while smoking 3–4 cigarettes per day), the formation and rupture risk will consequently be reduced (but not excluded). Indeed the majority of patients with cotinine levels of 0–1 have unruptured (32: 71%) vs ruptured aneurysm (13: 29%). Therefore, even a cotinine level of 1 cannot exclude either the presence of an aneurysm or its rupture.

Another unexpected result is that 72 nonsmokers from both groups had a cotinine level of 1 (10–30 ng/mL), instead of 0. This low cotinine level cannot be neglected. Among these, 23 patients had an aneurysm, and 8 of them a ruptured aneurysm. A cotinine level of 1 could result from secondhand smoke, but only 12/72 (17%) nonsmokers with a cotinine level of 1 claimed a possible home or work exposure to secondhand smoke. Continuous exposure to secondhand smoke increases the risk of developing cerebrovascular and cardiovascular diseases. Adams et al. studied the vascular endothelium of healthy active smokers, passive smokers, and control nonsmokers never exposed to secondhand smoke [1]. Using endothelial biopsy, they found that secondhand smoke reduces the activity of endothelial nitric oxide synthase and increases inflammation of the vascular endothelium in a way similar to that observed in active smokers, indicating the existence of direct toxic effects from secondhand smoke. Passive smoking and risk of rupture of an intracranial aneurysms have been evaluated in a retrospective study of 385 women with intracranial aneurysms [13]. Out of 282 nonsmokers with intact brain aneurysms, 67 (23.8%) were exposed to passive smoking, while among 75 nonsmokers with ruptured aneurysms, 19

(25.3%) were exposed to smoking (in the home or work environment). The authors concluded that there is no significant association between passive smoking and ruptured brain aneurysms.

It must be emphasized that a cotinine level of 1 could even be linked to diet and/or pollution. Low levels of nicotine are present in a number of frequently consumed vegetables, more specifically of the Solanaceae and Brassicaceae family (eggplant, bell peppers, chili peppers, potatoes, tomatoes, tomatillos, and cauliflower), as well tea leaves [18, 46]. On average, each fresh fruit of this vegetables contains nicotine at 2–7 μg/kg, and dietary sources may provide a maximum intake of 2.25 μg/day. Consumption of these plants for many years could explain the low cotinine level detected in our nonsmoker participants, who reported daily consumption of these foods. Another source of the low positive salivary or urinary cotinine level could be drinking water. Each year, many trillions of cigarette butts leaching nicotine are discarded, constituting one of the most abundant forms of waste in the world [30, 36, 51]. Cigarette butts containing nicotine pollute rivers and tap water and are strong indicators of anthropogenic contamination [43, 49]. Finally, it cannot be excluded that level 1 is the limit of the cotinine strip test in reliably identifying traces of the nicotine metabolite. Sampling of cotinine in the serum, as for other drug abuse, could better detect/determine low cotinine levels and could be routinely performed on a large scale.

Aging seems to influence cotinine levels. A study of 6423 cigarette smokers found a strong association between age and cotinine, independent of the number of cigarettes smoked per day [14]. Cotinine levels increased at each age group increment, peaking after age 40 but then declining among those aged more than 70 years. After considering some hypotheses, the authors suggested a prospective study to investigate the matter. In our study, cotinine levels declined significantly with increasing age, and there was a 2% reduction in cotinine level for each year of age. The reason could be reduced absorption of nicotine through the oral and respiratory mucous membranes. Malabsorption of many substances in the elderly, probably due to a reduction in absorptive mucosal surface or bacterial contamination, has been demonstrated [11, 32, 34, 52]. A lower risk of brain aneurysm formation and even rupture in elderly smokers may result.

Our study has a limitation. We did not collect samples within an hour of when the smokers last consumed nicotine, since we considered the cotinine result from the strip test to represent their daily consumption.

5. Conclusions

This study strongly supports the evaluation of cotinine levels in smokers with ruptured or unruptured brain aneurysms. High cotinine levels in smokers with brain aneurysm are significantly associated with high rupture risk, rather than aneurysm size. This examination should be routinely introduced as a large-scale screening tool in the prevention of brain aneurysm formation and rupture.

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Conflicts of interest/Competing interests:

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Code availability: Not applicable

Authors' contributions:

(1) the conception and design of the study, or acquisition of data, or analysis and interpretation of

data: Paolo Missori, Angela Ambrosone, Antonio Currà, Sergio Paolini, Giorgio Incarbone, Elena Amabile, Francesco Biraschi,

(2) drafting the article or revising it critically for important intellectual content: Paolo Missori,

Simone Peschillo, Sergio Paolini, Francesco Diana

(3) final approval of the version to be submitted: Paolo Missori, Angela Ambrosone, Antonio Currà, Simone Peschillo, Sergio Paolini, Giorgio Incarbone, Elena Amabile, Francesco Biraschi, Francesco Diana

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Figures

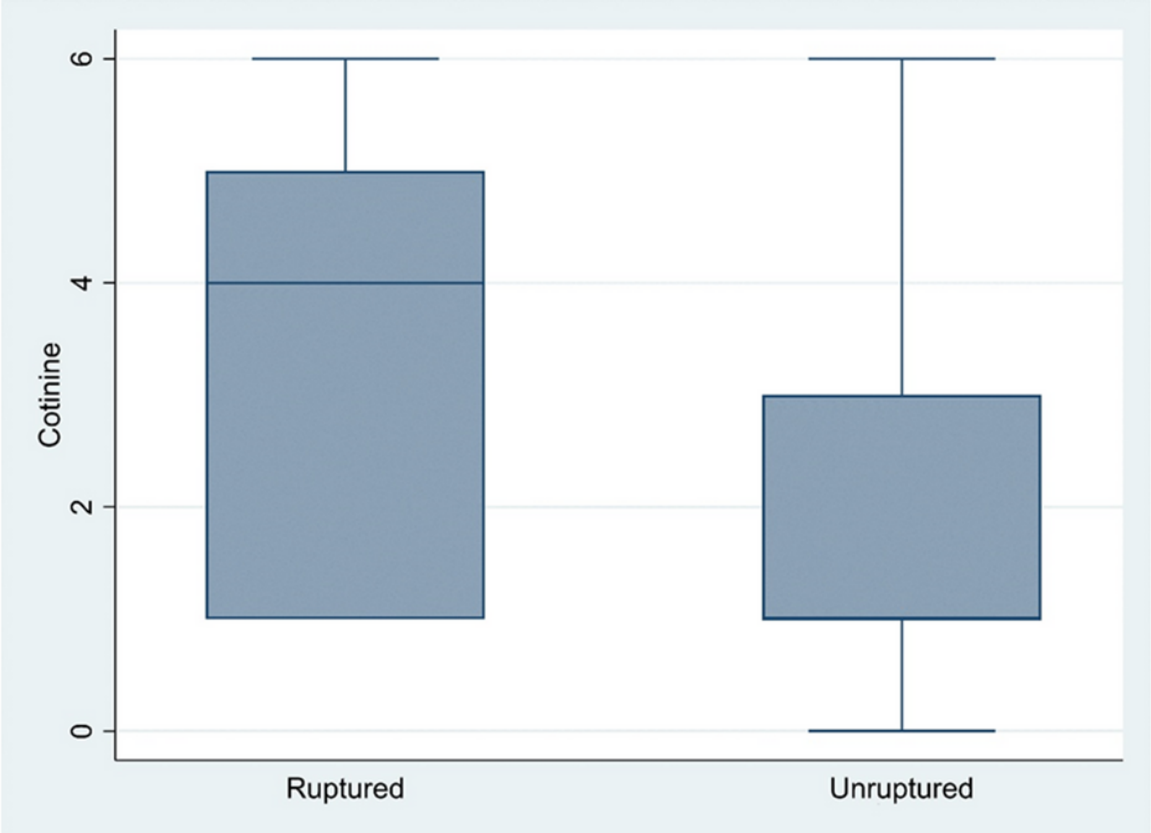

Figure 1

In smoker patients with brain aneurysm high cotinine level (≥4) is significantly associated to rupture.

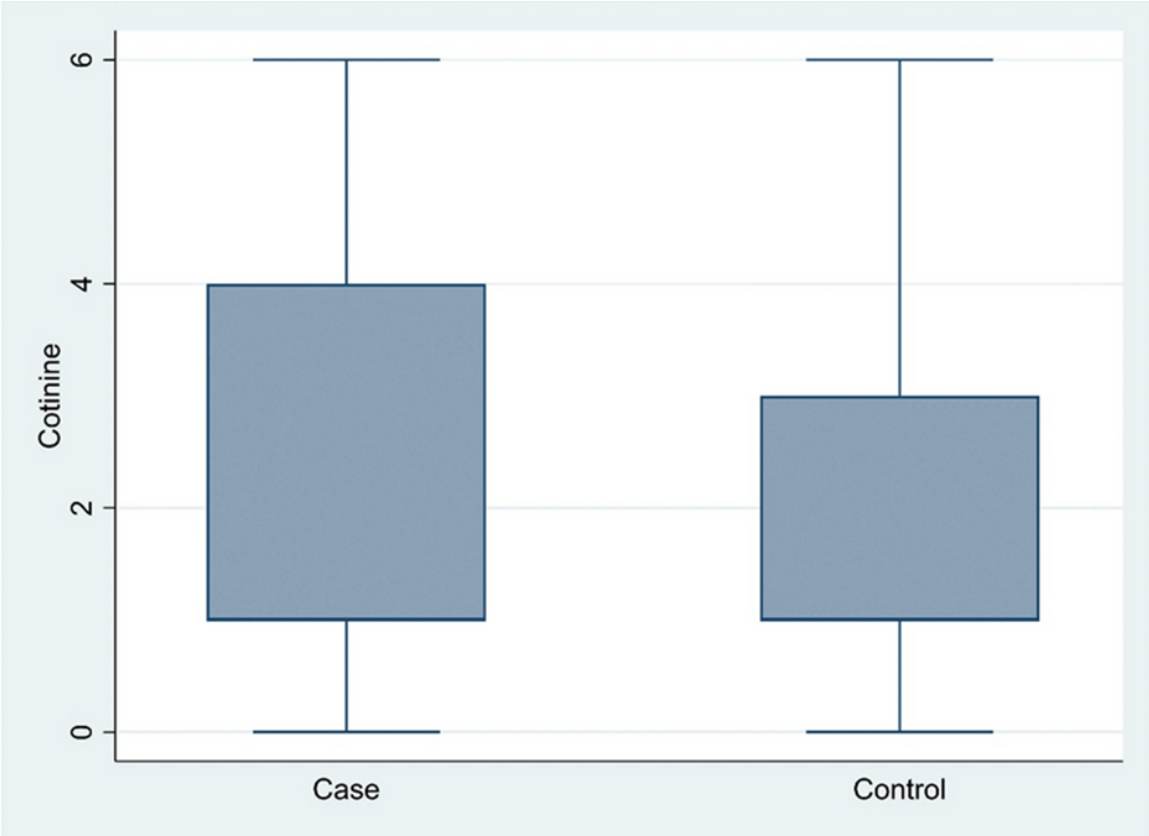

Figure 2

Cotinine level and aneurysm. There was a significant difference (P=0.009) in the distribution of cotinine between cases and controls.

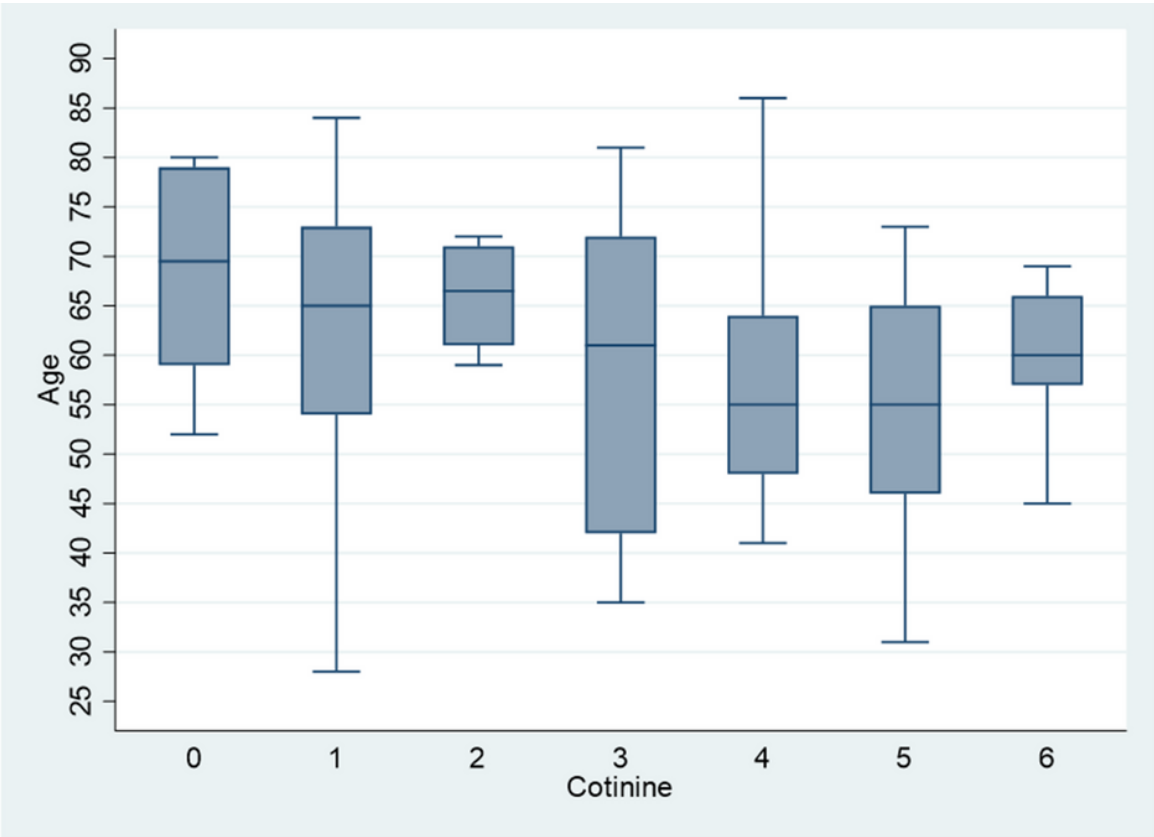

Figure 3

Cotinine level and age. There is a 2% decline in cotinine with each year of age.