



Characterization of inflammatory syndrome in smokers, from C-Reactive protein and erythrocytes sedimentation rate measurements

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Abstract

A local inflammatory syndrome is characterized by a classic semiological tetrad: pain, swelling, redness and heat. These signs are easily observed when the inflammation concerns the skin or an adjacent tissue. Throughout this study, the aim was to characterize chronic inflammation in smokers using two parameters, rate of erythrocytes sedimentation (RES) and C - reactive protein (CRP). Our study was done on a sample of 35 smoking subjects, composed of men and women. The CRP measurement was carried out using a CRP-Latex agglutination test which detects only serum CRP levels around 6mg/L. The technique used for the RES measurement is that of Westergreen. From the results, we observed that 31% of our sample presented a positive CRP and a high RES against 40% having regular CRP and RES. The gender of the subject did not play a role in the results obtained. On the other hand, a significant difference ($p = 0.031$) in CRP was observed between subjects with normal RES and those with high RES. Therefore, these results make it difficult to confirm that RES and CRP can be used as reliable markers for the characterization of inflammation linked to smoking.

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Introduction

Globally, smoking is one of the leading causes of preventable deaths and its proportion is constantly increasing (Lecours N *et al.*, 2012), it is recognized as a public health problem. The figures associated with smoking are very worrying and even alarming (Peltzer N, 2011). Already in Africa, the prevalence of smoking among adolescents is around 20% regardless of gender (Pampel F, 2008). In Gabon, it is believed that the prevalence in adults was around 19% in men and 3% in women in 2009 and the numbers are believed to be even higher today. These figs, admittedly unofficial, already should concern us, given the very harmful effects of smoking on the organism. In general, chronic smoking is the cause of a plethora of pulmonary disorders, cancer, cognitive disorders, proven cardio and cerebrovascular disorders (Kawada T, 2015). To this end, in addition to its addictive effects linked to nicotine, tobacco affects several tissues and organs according to its composition (Aldaham S *et al.*, 2015). These conditions can lead to chronic inflammation which could lead to cancer. Faced with this, numerous smoking markers are used in the biomedical field to provide objective measurements of the impregnation of tobacco. These markers used are components of tobacco smoke and their metabolites present in the body (Aldaham S *et al.*, 2015). These are generally nicotine, cotinine, produced by the transformation of nicotine in the liver, carbon monoxide (CO) or carboxyhemoglobin (HbCO). All these parameters are often characteristic of inflammatory processes. As a result, the rate of erythrocyte sedimentation (RES) and C-reactive protein (CRP) are markers of inflammatory conditions (Litao MK *et al.*, 2014) and have been widely used by clinicians in ambulatory and hospital settings (Buess T *et al.*, 1995). As part of our prospective study, we propose to characterize the inflammatory syndrome from the evaluation of these two biological markers of metabolism, the C-reactive protein (CRP) and the rate of erythrocyte sedimentation (RES), in adult smokers living in Libreville.

Material and methods

Study setting

This study was carried out at the National Public Health Laboratory in Libreville in Gabon.

Characteristics of the population of study

The subject likely to participate in the study must meet the following criteria: be over 15 years of age at the time of the study; be a regular smoker; have no lung infection or any other chronic illness; not being under medical treatment; be voluntary and accept the conditions of the study, answer the questionnaire by giving consent, accept the blood sample of a few milliliters.

Profile of the study

We conducted a prospective and transversal study. The prospecting lasted five months from March to August 2015.

Description of the study sample

There were 35 people total for the study, 30 men for 5 women, and their age ranged from 18 to 62.

Assessment of C-reactive protein (CRP)

The CRP measurement was carried out using a CRP-Latex agglutination test which detects only serum CRP levels around 6mg/L. CRP LATEX is a rapid latex test for the detection of Reactive Protein C (CRP). The latex particles, sensitized with antibodies specific to human CRP, are agglutinated in the presence of patient serum containing CRP. This reagent is supplied by BIOLABO SA, in France (Biomaghreb, 2007).

Evaluation of the rate of erythrocyte sedimentation (RES)

The method used to measure the rate of erythrocyte sedimentation (ESR) is that of (Beaudeau *et al.*, 2011). The result is read after one hour of reaction, taking into account the height of the plasma column above the red blood cells which have been deposited in an appropriate hemolysis tube containing 0.4 mL of 0.13 mol /citrate of sodium. The outcome of RES depends on the number, shape and volume of red blood cells and the plasma factors that alter the repulsion of red blood cells (NCCLS, 2000).

Statistical analyzes

The data was collected using/with Microsoft Excel and analyzed using Statview software 05. For the processing of these data, the comparison test was

used (unpaired t-test). The results are expressed as means plus or minus standard deviations and in percentage. The threshold of significance is expressed by p-value less than 0.05.

Results

Table 1 gives a general description of the sample. Women (14.29%) are less represented than men (85.71%). More than 42% of the subjects are between 29 and 39 years old. The average age of women is lower than that of men. In the population studied, more than half, or 54.29% smoked 2 to 14 cigarettes per day. The most popular brands are Dunhill® (58.82%), Marlboro Rouge® (31.43%), Fine Menthol® and Houston® (17.65%). Leaf tobacco (8.82%) is consumed less than cannabis (17.14%). They smoke more at home (88.57%) and at drinking places (80%) than at work (20%). The smokers in this study mostly ignore the chemical composition of tobacco (82.86%) and do not know well their effects on the organism (57.14%). Despite this state of affairs, 85.71% have expressed their intention to quit smoking, even if it is not known for how long (Table 1).

Concerning the individual motivations which push to smoke, the most cited are respectively to fight against stress, to feel and concentrate better. Except for one subject, the entire study population consumes alcoholic beverages. However, none of the individuals is undergoing specific treatment for an illness or infection and has not been the victim of any infection during the two weeks preceding the day of the blood test. With regard to clinical variables (CRP and RES), we note that a little more than half of our sample has a negative CRP (57.14%) and a weak RES (54.28%) (Table 1).

In the social parameters, there is a significant difference between the two sexes in the number of cigarettes smoked per day ($p < 0.05$). Men consume an average of 13.17 cigarettes versus 6 for women (Table 1).

On the other hand, for a 1st hour RES, it is high in subjects with positive CRP ($> 27 \text{ mm/h}$), even if this difference is not significant. This result indicates an obvious link between CRP and RESERS in smokers (Table 2).

Table 1. General characteristics of the population.

Variables	Workforce Percentage	
Genre (n=35)		
▪ Men	30	85.71%
▪ Women	5	14.29%
Âges (yrs, n=35)		
▪ 18-28	9	25.71%
▪ 29-39	15	42.86%
▪ >40	11	31.83%
Cigarette/day (n=35)		
▪ 2 - 14	19	54.29%
▪ >15	16	45.71%
Year for tobacco smocking		
▪ 1-25	29	82.86%
▪ >25	6	17.14%
Cigarette brand (n=35)		
▪ Fine menthol	6	17.65%
▪ Houston	6	17.65%
▪ Dunhill	20	58.82%
▪ Marlboro red	11	31.43%
▪ Marlboro light	4	11.76%
▪ Leaf tobacco	3	08.82%
▪ Cannabis	6	17.14%
Knowledge about the effects of tobacco (n=35)		
▪ Yes	15	42.86%
▪ No	20	57.14%
Intention to quit smoking (n=35)		
▪ Oui	30	85.71%
▪ Non	5	14.29%
Reason for smoking (n=35)		
▪ Pleasure	14	41.18%
▪ Morale	21	61.76%
▪ Stress	24	68.57%
▪ User-friendliness	5	14.71%
▪ Concentration	18	51.43%
Alcohol consumption (n=35)		
▪ Yes	34	97.14%
▪ No	1	02.86%
Infection in the past 2 weeks (n=35)		
▪ Yes	0	00.00%
▪ No	35	100%
Under medical treatment (n=35)		
▪ Oui	0	00.00%
▪ Non	35	100%
Place where we smoke (n=35)		
▪ Work	20	57.14%
▪ House	31	88.57%
▪ Drink establishment	28	80.00%
CRP enmg/L (n=35)		
▪ Without	20	57.14%
▪ With	15	42.86%
RES (mm/h,n=35)		
▪ High	16	45.71%
▪ Low	19	54.28%

RES: rate erythrocytes sedimentation, CRP: C-reactive protein.

Table 2. Variation of social and biological parameters according to gender, CRP and RES categories.

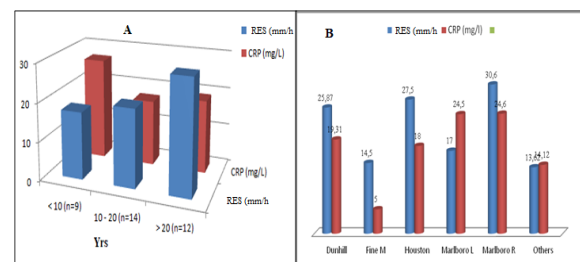
Social settings	Women (n=5)	Men (n=30)	p-value	CRP - (n = 20)	CRP + (n = 15)	p- value	RES - (n=18)	RES + (n=17)	p-value
Age (yrs)	34.25 ± 16.31	35.90 ± 10.91	ns	34.63 ± 12.18	37.06 ± 10.49	ns	36.38 ± 13.36	34.93 ± 8.98	ns
Nbr of years of smoking (yrs)	9.25 ± 5.96	16.63 ± 10.85	ns	14.18 ± 10.87	17.80 ± 10.26	ns	15.22 ± 12.96	16.37 ± 7.50	ns
Nbr of cigarette butt/day	6.00 ± 05.05	13.16 ± 7.45	0.046*	11.35 ± 7.98	13.20 ± 7.033	ns	12.44 ± 7.53	11.82 ± 7.77	ns
Biological parameters									
CRP (mg/L)	-	24.40 ± 30.41	ns				8.056 ± 15.11	28.64 ± 35.64	0.031*
RES (mm/h)	26.60 ± 20.81	22.03 ± 16.46	ns	19.20 ± 13.26	27.33 ± 20.30	ns			

The values presented are in the form of means ± standard deviation, *p < 0.05. Nbr of Cig / day: number of cigarettes smoked per day; Number of years of smoking: number of years of smoking, (-) undetectable thresholds. (ns): not significant.

The mean CRP for the group with a high RES is greater than 28mg/L compared to around 15mg/L for the other group. Which gives us a significant p-value (p = 0.03). The more time smokers spend smoking, the more their RES increases over the years. We go from a little over 17mm/h for people who have been smoking for less than 10 years to almost 30mm/h for those who have had the habit for over 20 years. On the other hand, the CRP seems to follow the opposite trend. The group of “new” smokers (<10 years) has the highest mean CRP (> 26mg/L). This value decreases for the other two groups (Fig. 1).

Smokers have been grouped according to the type of cigarette they smoke. The average age for all groups is between 32 and 39 years and that for the number of years of cigarette consumption is between 12 and 21 years. The group of subjects who consumed several cigarette brand smoke in average more cigarette than the rest. For the biological parameters,

the difference in mean CRP between the different groups remains very small. However, there is a significant difference when comparing the average RES first between the “Houston” group and the “more than one brand” group, then between the “Marlboro red” group and the “other” group”. For these two comparisons, we have respectively p = 0.002 and p = 0.02 (Table 3, Fig. 1B).

**Fig. 1.** Annual evolution (A) of CRP and RES and according to the brand (B) of cigarettes among smokers.**Table 3.** Variation in social and biological parameters depending on the type of tobacco consumed

	Dunhill®	Menthol L®	Houston®	Marlboro L®	Marlboro R®	Others®
RES (mm/h)	25.87 ± 21.37	14.50 ± 04.95	27.50 ± 03.53* ¹	17.00 ± 12.72	30.60 ± 16.93* ²	13.62 ± 03.81* ^{1,2}
CRP (mg/l)	19.31 ± 32.44	5.00 ± 00.00	18.00 ± 08.48	24.50 ± 33.23	24.60 ± 41.13	14.12 ± 21.25
Number of years of smoking (yrs)	15.46 ± 10.10	15.00 ± 14.14	18.00 ± 07.07	16.00 ± 08.48	21.00 ± 17.73	12.62 ± 08.24
Ages (yrs)	36.26 ± 12.10	33.00 ± 12.72	33.50 ± 06.36	32.50 ± 10.60	39.80 ± 14.70	34.12 ± 11.20
Number of cigarettes butt/day	12.50 ± 07.20	3.50 ± 00.70	06.50 ± 02.12	05.50 ± 02.12	14.00 ± 08.48	15.50 ± 08.00

The values presented are in the form of means ± standard deviation, * 1...2p < 0.05: statistically significant difference between the groups considered. Number of cig butt / day: number of cigarettes smoked per day; Number of years of smoking: number of years of smoking.

The strongest positive correlation is that of the duo, age / number of years of smoking ($r = 0.67$) with $p < 0.0001$, which is very significant. RES and CRP correlation remains no significant (Table 4, Fig. 2 & 3).

Tableau 4. distribution of correlations between different variables.

	Age (yrs)	Number yrs / smoking	RES (mm/h)	CRP (mg/L)	Number of cig butt/day
Age (ans)	1				
Years/tabac	0.67 (<0.0001)	1			
RES (mm/h)	0.62 (0.73)	0.15 (0.40)	1		
CRP (mg/L)	0.14 (0.43)	-0.04 (0.81)	0.32 (0.06)	1	
Number cig/day	-0.18 (0.31)	0.05 (0.79)	0.01 (0.98)	0.11 (0.53)	1

Number of cigarette butt/day: number of cigarettes smoked per day; Number of years / tobacco: number of years of smoking.

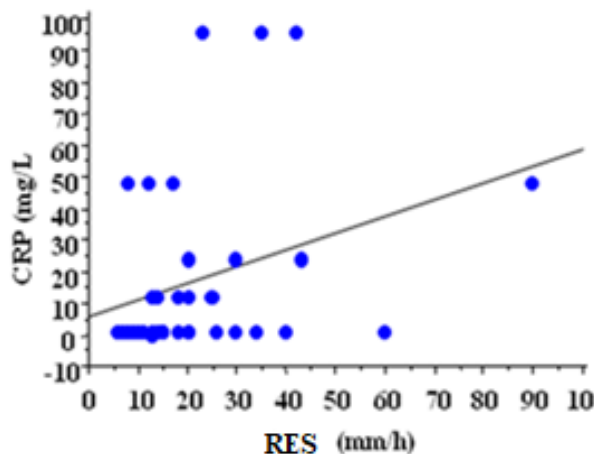


Fig. 2. The correlation between CRP and RES.

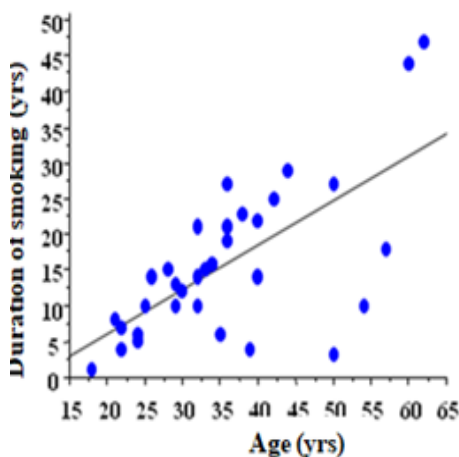


Fig. 3. The relationship between the number of years of smoking and age.

Discussion

The various observations that emerge from our main results are very characteristic of gender, age, socio-cultural behavior. The fact that there are fewer women who smoke or drink is not surprising in our community. The health statistical report (Pampel F, 2008) gives 19% of the prevalence of tobacco use among men aged 15 and over, among women in the same bracket it was 3% in Gabon in 2009 (Peltzer N, 2011). The reasons for trying to quit smoking are behavioral approaches to simply reassure oneself, and this is justified by the time taken to smoke, which exceeds 20 years for some.

For the number of cigarettes smoked per day, the averages of 1.36 and 5.63 were observed in men aged 15-28 and 29-79 respectively. For women, there were averages of 1.93 for the first age group and 2.73 for the second. This shows that the older you get, the more you need to smoke. Our results do not allow us to affirm that age, the number of years of tobacco consumption or the number of cigarettes consumed per day are parameters which directly and significantly influence the serum concentrations of CRP and RES. On the other hand, certain studies show an increase in the CRP reference values in smokers (Aldaham S *et al.*, 2015).

The aspect related to the brand of cigarette is also a real fact that emerges from this study. Because three brands stand out among themselves, the Marlboro red and the Dunhill and the Houston which present a significant RES compared to the other brands of cigarettes.

In the results obtained, just over 31% have a positive CRP and a high RES. These proportions therefore do not allow us to conclude that smoking leads to a significant increase in inflammatory markers, even if the trend exists, while some studies have clearly established this link (Asthana A *et al.*, 2010). In fact, the CRP is higher for consumers with a shorter duration of consumption, while for RES the values are important for long-term consumers. It could be suggested that CRP is a marker for acute inflammatory disease while RES is a marker for chronic inflammation (Olivier Chenillot, 2000).

Study notes that CRP and RES are higher among regular smokers versus non-smokers (Ohsawa M *et al.*, 2005). They concluded that cigarettes are a contributing factor in increasing CRP and RES. According to one study, the increase in CRP remains after the subject has stopped smoking (Ohsawa M *et al.*, 2005). In our study, we observed cases where the subjects had a positive CRP accompanied by a normal RES and others where the CRP was negative and the high RES. Even if these variations seem very random, the fact remains that long-term smoking could lead to an adaptation of the immune system in certain subjects whose sensitivity becomes almost zero. Indeed, over time, the nature of the cigarette, the frequency, even the inhalation technique would be integrated as usual behavior. It is in this sense that Aldaham and collaborators define in their study an inflammatory reaction resulting from the action of the particles contained in the smoke of a cigarette on the mucous membrane of the respiratory tract like a chronic inflammatory reaction (Aldaham S *et al.*, 2015).

Conclusions

The harmful action of smoking on the body is no longer to be demonstrated. Inflammation of the nasal lining, mouth, lining of the bronchi or bronchioles is characteristic of smoking. In our context, it involved measuring CRP and RES in smokers, to indirectly estimate the degree of the inflammatory syndrome. Given the size of our sample limited to only 35 patients and the period of this study, the two parameters show opposite behaviors as a function of time and according to the brand of cigarette. Indeed, CRP is a marker of acute inflammation while RES is more linked to chronic inflammatory smoking. There is a strong relationship between certain cigarette brands and the RES, where the Marlboro brand stands out very significantly.

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