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Full Length Research Paper

Profile of CA 15-3 and CEA during breast cancer chemotherapy at Ouagadougou, Burkina Faso

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Biomarkers are considered as an innovative tool in the diagnosis and follow-up of breast cancer. The aim of the study was to assess the profile of circulating tumour markers CA 15-3 and CEA in patients under chemotherapy for breast cancer in Ouagadougou. This is a prospective cross-sectional study with a descriptive and analytical aims which was done from July to November 2020. Patients with histologically confirmed malignant breast tumour and under chemotherapy were included. Results revealed that the study was on thirty (30) female patients whose average age was 47.47 ± 2.10 years with a mean BMI of 27.29 ± 1.09 kg/m^2. It was a non-specific type of infiltrating carcinoma with SBRm II grade in 90% of the patients. The mean CA 15-3 was 212.98 U/mL before chemotherapy and 165.75 U/mL after it. The CEA mean value was 3.13 ng/L before chemotherapy and 16.14 ng/L after it. Serum CA 15-3 was significantly associated with tumour site, SBRm grade, chemotherapy line and treatment response. Serum CEA level was significantly associated with tumour site and SBRm grade. Despite their lack of sensitivity, tumour markers, particularly CA 15-3 enabled assessment of the response to treatment in patients in this study.

Key words: Tumour markers, CA 15-3, CEA, breast cancer, chemotherapy.

INTRODUCTION

In Burkina Faso, breast cancer is responsible for 17.7% of cancer-related deaths in women. The treatment of this cancer is multidisciplinary and is often based on a strategy combining chemotherapy, surgery, radiotherapy, hormone therapy and/or targeted therapy. In Burkina Faso, chemotherapy plays an important role and is an

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integral part of care of patients suffering from all types of cancer. It constitutes an important component of effective care for breast cancer: given to patients at an early stage, it has a good impact on survival and contributes to cure. However, once treatment fails, patients' quality of life and survival rate are significantly affected. Therefore, it is essential to identify reliable prognostic factors to guide decision-making during the treatment of breast cancer in order to improve prognosis. This is how treatment has made great strides over the past decades with the discovery of prognostic biomarkers which make possible the use of individualized treatments. The concentration of circulating marker detected in the biological fluid is an indirect estimator of tumour mass or tumour aggressiveness; thus, allowing the assessment of tumour progression and/or therapeutic efficacy (Uygur and Gümüş, 2021).

Of all serum tumour markers for breast cancer, CA 15-3 and CEA were most used and recommended (Ashour Byomy et al., 2021; Imran et al., 2021; Khushk et al., 2021; Uygur and Gümüş, 2021). The European Group on Tumour Markers recommended that CEA and CA15-3 levels should be used for prognosis assessment, early detection of disease progression and monitoring of breast cancer treatment (Cardoso et al., 2019). While some authors suggested routine testing of tumour markers, the systematic use of serum markers in the strategies of women follow-up after breast cancer treatment is excluded from international main guidelines (Moschetti et al., 2016).

With these controversies in the monitoring of breast cancer patients, we therefore wanted to focus on the measurement of CA 15-3 and CEA in patients with a malignant breast tumour and treated by chemotherapy at Ouagadougou (Burkina Faso), with the aim of contributing to an early detection of metastases and/or therapeutic failure.

MATERIALS AND METHODS

Study context, type and period

This was a prospective cross-sectional study with descriptive and analytical aims led from July to November 2020. Patients were recruited at the University Hospital Centre of Bogodogo and at Sandof Polyclinic in Ouagadougou, Burkina Faso. Serum marker assays were performed in the laboratory of Sandof Polyclinic.

Samples

The tests were performed on the patients' serum, after venous blood sampling on dry tube. For the pre-therapeutic assays, samples were taken before the first course of chemotherapy. The results of these pre-therapy assays were extracted from the patients' files.

For the post-therapy samples, we took the samples at the end of the patients' last chemotherapy treatment (six-treatment protocols). The samples were centrifuged at 3500 rpm for 5 min, then the serum was aliquoted and stored at -80°C until analysis (Farahani et al., 2020).

Approach

Sampling was comprehensive during the period of study. Patients were selected in collection centres after file study among patients under chemotherapy for breast cancer and meeting the following criteria: histologically confirmed breast malignancy; patients with at least four courses of treatment allowing assessment of chemotherapy efficiency; complete clinical record; being aged at least 18 years and have given their free and informed consent to participate in the study. Patients had to be undergoing adjuvant or neoadjuvant chemotherapy to be included in the study and those undergoing palliative chemotherapy were not included. Patients who were not clinically and/or radiologically assessed for progression on chemotherapy were not also included.

Socio-demographic characteristics were obtained by interviewing the patients. Clinical and histological data were obtained from patients' records, consultation and hospitalization registers. Evaluation of hormone receptor status, HER2 expression and quantification of the Ki67 proliferation index were performed by immunohistochemical techniques, and the results were extracted from the patients' files. The immunohistochemical study was performed on paraffin sections by manual technique, using the Ultravision Quanto detection system kit, with DAB (Shi et al., 1999).

CA 15-3 and CEA Serum concentrations were determined in patients' serum using VIDAS® CA 15-3 (153) kit, reference 30429-01 and VIDAS® CEA(S) (CEAS) kit, reference 30 453-01, on the Biomérieux® Minividas automated system by ELFA technic (Enzyme Linked Fluorescent Assay) (Deliu et al., 2018; Abed et al., 2020). The normal serum values retained for CA15-3 and CEA were respectively < 30 U/mL and < 5 ng/mL.

For the study, clinical tumour response and imaging response (RECIST (Dubreuil et al., 2017) assessed by the oncologists and available in the patients' files were used and compared with the results of tumour marker assays.

Tumour response was considered as good in patients with complete or partial remission and poor when the patient was in stabilization or progression.

All study data were entered in Excel and analyzed using Stata version 13.0 software. Student T test was used to compare the averages between the different groups. Statistical tests were considered significant when p was less than 0.05.

The study was approved by the institutional Ethics Committee of Saint Camille CERBA (Pietro Annigoni Biomolecular Research Centre), reference N° 2020/II-03-016. Authorisation for data collection was obtained from the management of each collection centre. Data confidentiality was maintained throughout the study.

RESULTS

A total of thirty (30) patients were included in the study; ten (10) patients at the University Hospital Centre of Bogodogo and twenty (20) at Sandof Polyclinic.

Socio-demographic characteristics

The mean age of the patients in the study was 47.47 ± 2.10 years; with extremes ranging from 33 to 74 years. 16 patients (53.33%) were under 45 years old and 14 (46.67%) were over 45 years old. The average body mass index (BMI) was 27.29 ± 1.09 kg/m^2 (ranging from 13.76 to 39.67 kg/m^2). Of the patients, 11 (36.67%) had a normal BMI, while 19 (63.33%) were overweight or obese. Two patients (6.67%) had a personal history of

Table 1. Distribution of patients according to clinical and histological characteristics.

Parameter	Characteristics	Number (n=30)	%
Location of tumour	Right breast	18	60.00
	Left breast	10	33.33
	Bilateral	2	6.67
Location on breast (n=21)	QSE	18	60.00
	Other locations	12	40.00
Tumour size (T)	T2	7	23.33
	T3	1	3.33
	T4	13	43.34
	Tx	9	30.00
Number of lymphadenopathy (N)	N0	3	10.00
	N1	17	56.67
	N2/N3	2	6.66
	Nx	8	26.67
Metastases (M)	M0	19	63.33
	M1	11	36.67
SBRm	II	27	90.00
	III	3	10.00
Histological type	NSIC*	27	90.00
	DCIN**	2	6.67
	ILC***	1	3.33

* Non-specific infiltrating carcinoma **Ductal carcinoma in situ ***Infiltrating lobular carcinoma.

breast cancer and four (13.33%) had a family history of breast cancer.

Clinical and histological characteristics

Tumour damage was predominant on the right breast of 18 patients (60%). Tumour mass was present in the upper-external quadrant in 60.00% of patients and the histological grade SBRm II was the most found (90%). The majority of women were on their first line chemotherapy (70%) and metastases were present in 36.67%, mainly bone metastases.

The distribution of patients according to clinical and histological characteristics is presented in Table 1.

Immunohistochemical characteristics

In the population studied, 13 patients (43.33%) were able to perform hormone receptor (ER and PR) evaluation: 11 (84.62%) were positive and 2 (15.38%) were negative. Moreover, 10 patients (33.33%) were tested for HER2 protein and Ki67 antigen. All HER2 results were negative; four patients (40%) had Ki67 ≤ 25% and six (60%) had Ki67 > 25%. Thus, we got 84.62% luminal A tumours and 15.38% triple negative tumours.

Chemotherapeutic characteristics

The majority of patients (70%; that is 21 patients) were on first-line chemotherapy, while nine (9) patients (30%) were at least on second-line chemotherapy. The main protocol used was FAC (5 Fluorouracil, Adriblastine, Cyclophosphamide) administered to 56.67% of patients. Fourteen (14) patients (46.67%) had a satisfactory response to chemotherapy assessed by clinical and/or imaging studies, while sixteen (16) (53.33%) had a poor response.

Circulating tumour markers

CA 15-3 was measured in 12 patients (40%) before the start of chemotherapy, while CEA was available in 7

Table 2. Pre- and post-treatment concentrations of tumour markers.

Tumour marker		Average	Standard deviation	p-value	Minimum	Maximum
CA 15-3 (U/mL)	Pre-therapeutic (n=12)	212.98	180.28		8.30	2194.21
	Post-therapeutic (n=12)	137.69	57.11	0.677	13.75	570.84
	Post-therapeutic (n=30)	165.75	76.50	0.136	9.30	2273.32
CEA (ng/L)	Pre-therapeutic (n=7)	3.13	0.38		2.19	4.87
	Post-therapeutic (n=7)	5.31	4.04	0.597	1.62	29.48
	Post-therapeutic (n=30)	16.14	10.90	0.357	0.66	324.43

Table 3. Post-therapeutic changes of CA 15-3 and CEA according to the epidemiological characteristics of patients.

Parameter	Characteristics	CA 15-3		CEA	
		Mean U/mL	p-value	Mean ng/L	p-value
Age	≤ 45 years (n=16)	44.76±09.02	0.091	1.59±0.39	0.157
	> 45 years (n=14)	304.04±158.46		32.77±22.97	
BMI	Normal (n=11)	172.78±50.20	0.946	11.23±6.35	0.738
	High (n=19)	161.69±118.63		18.99±16.97	
PH* of breast cancer	Yes (n=2)	38.08±13.44	0.663	1.03±0.11	0.718
	No (n=28)	174.87±81.78		17.22±11.66	
FH** of breast cancer	Yes (n=4)	23.11±5.89	0.474	3.78±2.23	0.664
	No (n=26)	187.70±87.68		18.04±12.56	

PH*: personal history FH**: family history.

patients (23.33%). The mean concentrations of tumour markers drawn from the patients' records (pre-therapeutic CA 15-3 and CEA) and those obtained from our assays (post-therapeutic CA 15-3 and CEA) are shown in Table 2. Subsequently, the mean CA 15-3 and CEA values before chemotherapy were 212.98±180.28 U/mL and 3.13±0.38 ng/L respectively. Of the 12 patients with pre-therapy CA 15-3, five had high values and seven had normal values. For the pre-therapeutic CEA, all seven patients had normal values. After chemotherapy, the mean CA 15-3 was 165.75±76.50 U/mL and the CEA was 16.14±10.90 ng/l.

Study of the variation of post-therapeutic CA 15-3 and CEA according to patients' characteristics

Tables 3, 4 and 5 summarize post-treatment variations of CA 15-3 and CEA respectively according to the epidemiological, clinical and histological, immune-histochemical and chemotherapeutic characteristics of the patients. Thus, the markers showed statistically significant variations depending on the site of the tumour, the presence of metastases, SBRm grade, chemotherapy line and response to treatment.

DISCUSSION

The objective of the study was to assess the profile of circulating markers CA 15-3 and CEA in patients under chemotherapy for breast cancer at Ouagadougou. The main limitation of the study is the size of the sample obtained, which does not allow the conclusions to be extrapolated to the entire population of patients treated for breast cancer. Only 40 and 23.33% of the patients had respectively benefited from CA15-3 and CEA tests before treatment. Although these biomarkers are not recommended for cancer screening, diagnosis or staging (Harris et al., 2007), the interest of pre-therapeutic initial values is clearly established, especially for a comparison with later figures. Indeed, the interest of measuring their levels before any treatment is to have an individual reference value which is essential to assess the effectiveness of a treatment and/or to carry out a later monitoring. The detection of a biological recurrence is

Table 4. Post-therapeutic changes of CA 15-3 and CEA according to clinical and histological features.

Parameter	Characteristics	CA 15-3		CEA	
		Mean U/mL	p-value	Mean ng/L	p-value
Location of tumour	Unilateral (n=28) Bilateral (n=2)	85.56±24.41 1288.50±984.82	0.000	5.60±2.58 163.73±160.69	0.000
Location on breast	QSE (n=18) Others (n=12)	220.50±125.62 83.63±31.07	0.390	23.61±18.07 4.93±2.59	0.411
Tumour size (T)	T2/T3 (n=8) T4/Tx (n=22)	173.75±74.92 162.85±101.68	0.951	14.44±8.51 16.76±14.66	0.927
Number of lymphadenopathy (N)	N0/Nx (n=11) N1/N2/N3 (n=19)	47.19±26.46 234.40±118.10	0.245	4.33±2.86 22.98±17.10	0.419
Metastases (M)	M0 (n=19) M1 (n=11)	53.97±18.07 358.83±198.45	0.026	2.45±0.56 39.79±29.16	0.099
SBRm	II (n=27) III (n=3)	98.82±26.24 768.15±752.59	0.003	5.83±2.67 108.97±107.73	0.001
Histological type	NSIC (n=27) Others (n=3)	182.88±84.50 11.62±1.20	0.511	17.80±12.09 1.26±0.28	0.657

Table 5. Post-therapeutic changes of CA 15-3 and CEA according to chemotherapeutic immunohistochemical characteristics.

Parameter	Characteristics	CA 15-3		CEA	
		Mean U/mL	p-value	Mean ng/L	p-value
Hormone receptors	Yes (n=13) No (n=17)	277.16±172.55 80.56±24.41	0.208	34.65±24.74 1.99±0.40	0.140
Immunohistochemical classification	Luminal A (n=11) Triple negative (n=2)	320.29±202.51 39.96±11.55	0.580	40.68±29.06 1.50±0.58	0.590
Ki67	≤ 25 % (n=4) > 25 % (n=6)	724.87±531.79 107.56±50.75	0.185	98.86±76.79 8.74±5.01	0.178
Chemotherapy line	1st line (n=21) Multiple lines (n=9)	68.28±20.65 393.20±242.87	0.024	3.63±1.55 45.33±35.63	0.079
Response to chemotherapy	Good (n=14) Poor (n=16)	24.51±3.77 289.35±137.92	0.042	1.67±0.44 28.80±20.19	0.220

earlier if one refers to the basal value of each patient rather than to a single statistical threshold (Yoo et al., 2021).

The mean pre-therapeutic CA 15-3 was 212.98 U/mL (Table 2), well above the normal value (<30 U/mL). On the other hand, the mean pre-therapeutic CEA was 3.13 ng/L (Table 2); all patients had a normal value < 5 ng/L. The clinical significance of preoperative serum levels of CEA and CA 15-3 in breast cancer remains controversial. Indeed, Molina et al. (2010) found abnormal serum levels of CEA (> 5 µg / L) or CA 15.3 (> 30 kU / L) respectively in 12.7 and 19.6% of their patients. Serum concentrations of CEA and CA 15-3 were clearly linked to tumour size and lymph node damage, with significantly higher

concentrations in large size tumours and those with lymph node damage (Molina et al., 2010). The lack of sensitivity and specificity of CEA led the expert groups to not recommend its measurement in the screening and diagnosis of carcinomas of various locations. Even in the initial assessment, the value of its measurement remains debated at the international level and some experts do not recommend it because it does not modify the therapeutic attitude (Durand and Beaudeux, 2011).

Post-therapy CA 15-3 and CEA values were 137.69 U/mL (n=12) and 5.31 ng/L (n=7), respectively, a decrease in the mean value for the former marker and an increase for the latter, but not statistically significant (Table 2). Of the patients with normal CA 15-3 before chemotherapy, five (5) maintained values below 30 U/mL, as did CEA, which remained normal. These pre-therapeutic markers allowed us to infer a good prognosis which was confirmed by the clinical course of the patients. Many authors highlighted the correlation between the evolving profile of CA 15-3 and the response to treatment, and various recommendations stipulate that an initial elevation of CA 15-3 which does not return to the normal reflects a lack of response to treatment and constitutes an important unfavourable prognosis factor (Bushi and Trebicka, 2021). No significant variation of tumour markers was found based on epidemiological characteristics in the study (Table 3). However, analysis of marker variations based on clinical and histological characteristics revealed a significant association with tumour site and SBRm grade for both markers; and with presence of metastases for CA 15-3 (Table 4). We did not find a significant association of CA 15-3 and CEA values with the immunohistochemical characteristics of the patients (Table 5). Li et al. (2018) did not also find any difference of serum marker levels based on immunohistochemistry. On the contrary, other studies showed that CA15-3 levels differ significantly according to molecular subtype (Li et al., 2020; Ruswendro et al., 2021).

Variations of CA 15-3 were statistically significant according to patients' treatment line and response to chemotherapy; whereas the mean values of CEA showed no statistically significant variation according to these characteristics (Table V). Indeed, patients with a poor response to chemotherapy, as well as those who were at least at their second line had higher CA 15-3 values (Table V). The pattern was similar for CEA even if the variations were not statistically significant (Table 5). Then, CA 15-3 values were clearly linked with prognosis in patients and predicted response to treatment in our patients. The prognostic value of CA15-3 had been proven by some studies (Gonssaud et al., 2017; Li et al., 2018; Uygur and Gümüş, 2021), while other studies reported negative results (Rasmy et al., 2016). For Ebeling et al. (2002) in a study of 1046 patients, CA15-3 in univariate analysis but not in multivariate analysis was predictive of a poor outcome. In a review paper, Duffy (2006) collected at least 10 studies and reported in a descriptive way that higher CA15-3 may be associated with a poor outcome, but he did not perform a pooled analysis to confirm the results. The reason why CA15-3 can predict breast cancer prognosis is not very clear, but as CA15-3 is the soluble form of MUC1, this may be related to the function of MUC1. It has been reported that MUC1 does not only allow cancer cells to escape the immune system, but also promotes cancer cell migration by activating certain membrane receptors (Oral et al., 2020; Khodabakhsh et al., 2021). CEA is less widely studied as a prognostic factor than CA15-3 because it is less positive and more controversial. Some studies reported that CEA does not allow to distinguish primary from metastatic breast cancer (Ebeling et al., 2002; Molina et al., 2010; Nan et al., 2017), but others reported that high CEA levels were associated with a poor prognosis of breast cancer (Li et al., 2018; Imran et al., 2021; Ashour Byomy et al., 2021). These conflicting results of CA15-3 and CEA in breast cancer with respect to their prognostic value may be due to small sample sizes, variable study designs or other biases in each study. At present, the use of serum tumour markers in breast cancer is poorly established due to their low sensitivity and specificity. Many studies reported low positive CA15-3 and even lower CEA (Shao et al., 2015; Wu et al., 2014). Without more potent serum markers, although imperfect, CA15-3 and CEA remain the most commonly used biomarkers in breast cancer and are recommended for practical use by the American Society of Clinical Oncology (ASCO) (Harris et al., 2007). Likewise, the European Group on Tumour Markers recommended the use of CA15-3 and CEA to assess the prognosis of breast cancer (Duffy et al., 2017).

Conclusion

As the most common malignancy in women, breast cancer is a great threat for women's health worldwide. Its treatment by chemotherapy requires relevant clinical, radiological and biological evidence to assess the response to treatment. Our study allowed a quantitative assessment of tumour markers CA 15-3 and CEA. The main limitations of the study are the size of the population and the fact that tumour markers were measured in a limited number of patients before chemotherapy. Our results suggest that the levels of these markers, particularly CA 15-3, may be useful in predicting the prognosis of breast cancer in patients. As the examination of these markers is still not widely used in daily clinical practice, the data obtained provided important information for identifying patients with a poor response to chemotherapy. However, individual biological monitoring should be ensured by a single laboratory and a single technique. Interpretation of the levels of these markers must take into account their evolutionary

profiles, but also the clinical and radiological conditions of the patients.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Association of hyperuricemia and metabolic syndrome in type 2 diabetes mellitus patients in Dakar

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The association between hyperuricemia and metabolic syndrome (MS) has been reported in many studies. The authors performed this cross-sectional study to determine the association between hyperuricemia and the MS among diabetic patients in Dakar. Type 2 diabetic patients received as part of their follow-up at the Marc Sankalé Center of Abass Ndao Hospital in Dakar were enrolled. For each patient, blood samples and 24 h urine collection were performed. Hyperuricemia was defined for uric acid concentrations > 416 µmol/l in men and > 357 µmol/l in women and the MS was evaluated according to WHO criteria. Statistical analysis was done using the XLSTAT 2019 software. A total of 153 type 2 diabetic patients were included with an average age of 56.63 years. Thirty-one percent (31%) of patients had metabolic syndrome and 32% of them had hyperuricemia. Significant correlations were found between serum uric acid and some components of the MS including triglyceride levels (r = 0.25, p = 0.002), microalbuminuria (r = 0.19, p = 0.018), and fasting glucose (r = - 0.22, p = 0.005). The authors found that hyperuricemia is frequent in patients with MS and this could be considered as a biomarker associated with the presence of this syndrome.

Key words: Hyperuricemia, metabolic syndrome, type 2 diabetes, uric acid.

INTRODUCTION

Metabolic syndrome (MS) is a known risk factor for many chronic diseases including type 2 diabetes mellitus, cardiovascular diseases (CVD), chronic kidney diseases (CKD), among others (Lee and Sanders, 2012).

The World Health Organization (WHO) defines MS by the presence of insulin resistance [e.g. type 2 diabetes (T2D) or indications of abnormal glucose metabolism], together with at least two of the following factors: use of anti-hypertensive medication and/or high blood pressure (BP) ≥ 140 mmHg systolic or ≥ 90 mmHg diastolic],

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plasma triglycerides >150 mg/dL, HDL cholesterol < 35 mg/dL in men or < 39 mg/dL in woman, body mass index (BMI) > 30 kg/m^2 and/or waist-hip ratio >0.9 in men, > 0.85 in women, and urinary albumin excretion rate ≥ 20 µg/min or albumin creatinine ratio (UACR) ≥ 3.4 mg/mmol (Alberti and Zimmet, 1998). It is a real public health problem around the world, with a frequency and a prevalence increasing in both developing and developed countries (ViswanathanI and Deepa, 2006). Serum uric acid (SUA) is the end product of purine metabolism in humans and many studies have reported the association between hyperuricemia and the various components of the MS, in particular obesity, blood pressure, hyperlipidemia as well as glucose intolerance (Ames et al., 1981; Wilson et al., 2005; Lorenzo et al., 2007; Fabbrini et al., 2014; Zhang et al., 2016; Cheserek et al., 2018; Huang et al., 2020).

Indeed, epidemiological studies have suggested that uric acid is a risk factor for cardiovascular disease and in the MS a high frequency of hyperuricemia is found which would be a compensatory mechanism to counteract the oxidative stress found in the circumstances of this syndrome (Hansel et al., 2004; Sung et al., 2004; Ishizaka et al., 2005; Ismail et al., 2018). Thus, the authors carried out this study with the main objective of determining the association between uricemia and metabolic syndrome in a population type 2 diabetics.

PATIENTS AND METHODS

Study design and subjects

It was a cross-sectional and prospective study conducted over 7 months from March to September 2018. This study was carried out on type 2 diabetics received in consultation as part of their follow-up at the Marc Sankalé Center of Abass Ndao Hospital in Dakar.

The study was approved by the Scientific Ethics Committee of the Faculty of Medicine, Pharmacy and Odontology of the Cheikh Anta Diop University of Dakar and informed consent was also obtained from patients.

Patients with conditions or taking drugs that could interfere with uric acid levels as well as those who did not express their consent to participate in the study were not included. In this study, the MS was assessed according to the WHO criteria (Alberti and Zimmet, 1998).

This definition includes a state of diabetes mellitus or a fasting blood sugar ≥ 110 mg/dl (6.10 mmol/l) in addition to two of the following features:

1) A waist / hip ratio > 0.90 for men and > 0.85 for women or a BMI ≥ 30 Kg /m2;
2) A triglyceride level > 150 mg/dl (1.7 mmol/l) and / or HDL-C < 35 mg/l (0.9 mmol/ l) for men and < 39 mg/dl (1.0 mmol/l) for women;
3) A blood pressure > 140/90 mm Hg or an antihypertensive treatment;
4) and microalbuminuria > 30 mg/24h.

Data collection

The epidemiological data were collected using a questionnaire and for each patient, blood samples were taken after 12 h of overnight fasting by venipuncture at the bend of the elbow. A 24 h urine collection was also performed for the determination of microalbuminuria.

The blood samples were centrifuged at 3000 revolutions/min for 5 min and were immediately processed or stored at -20ºC until use. All biochemical variables, except HbA1c, were measured using Cobas 6000 / c501® analyzer (Roche, Hitachi, Germany) following the protocol provided by the reagent manufacturer and glycated hemoglobin (HbA1c) was measured using D-10® system (BioRad, USA).

Uric acid was determined by the uricase enzymatic method with quantification of the hydrogen peroxide formed by a Trinder reaction.

The body mass index (BMI) was defined as weight in kilograms divided by the square of the height in meters. The blood pressure (BP) was measured in a sitting position by using a standardized automatic electronic sphygmomanometer.

Hyperuricemia has been defined for uric acid concentrations > 416 µmol/L in men and > 357 µmol/L in women (Hochberg et al., 2003).

Statistical analysis

Statistical analysis was performed using XLSTAT 2019 software. Data were presented as frequencies and percentages for categorical variables and as the mean ± SD for continuous variables. All continuous variables were tested for normal distribution by Shapiro–Wilk test, and the significance of differences between groups was tested with an unpaired t-test and/or Mann–Whitney U-test**.** Categorical variables were compared using the Chi-squared test and the association between the variables was evaluated using the Spearman correlation test. A p value less than 0.05 were considered significant.

RESULTS

A total of 153 type 2 diabetic patients including women (65%) and 54 men (35%) were enrolled. Patients were aged between 21 and 87 years with an average of 56.63 years. The mean duration of diabetes was 8.28 ± 6.54 years. In the study population, 31% of patients had metabolic syndrome and were characterized by a predominance of women (Figure 1).

The general characteristics of the study population according to the serum uric acid concentrations are illustrated in Table 1. they found that 32% of patients with metabolic syndrome had hyperuricemia (Figure 2).

The study of the correlations between uricemia and the various components of metabolic syndrome revealed significant positive correlations between serum uric acid and various parameters such as triglyceride ($r = 0.25$; $p = 0.002$) and microalbuminuria ($r = 0.19$; $p = 0.018$). The authors also found significant negative correlations between uricemia and blood sugar as well as glycated hemoglobin levels with respectively ($r = -0.22$; $p = 0.005$) and ($r = -0.25$; 0.002). No significant correlations were found for the other parameters ($p > 0.05$) (Table 2).

DISCUSSION

Metabolic syndrome consists of the association in the

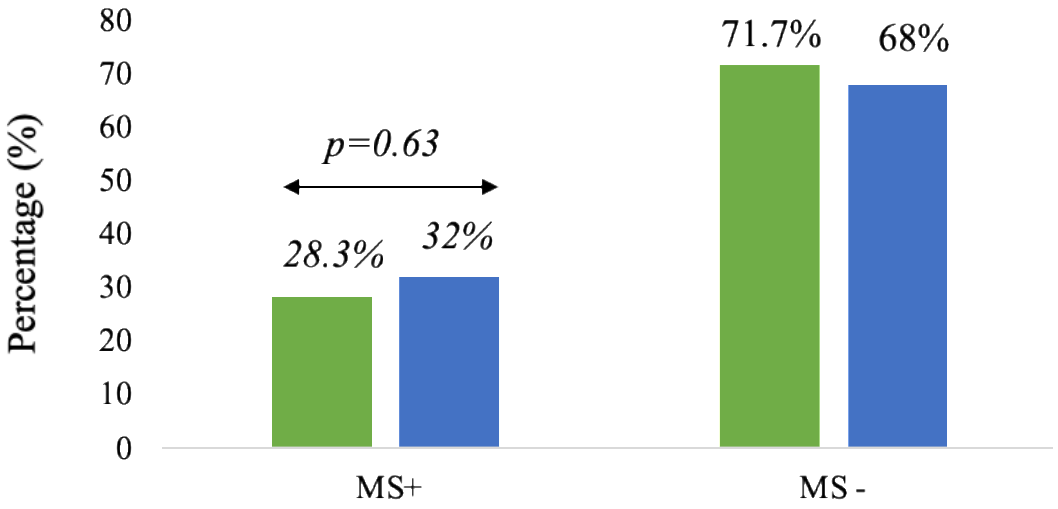

Figure 1. Frequency of metabolic syndrome according to sex. MS +: patients with metabolic syndrome, MS -: patients without metabolic syndrome, M: males, F: females.

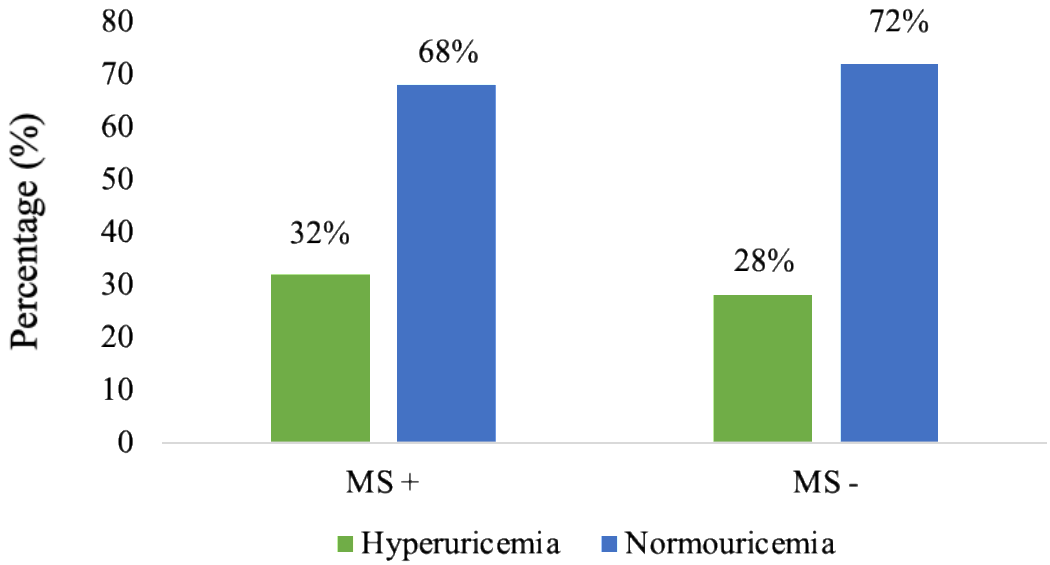

Figure 2. Frequency of hyperuricemia in patients with and without metabolic syndrome. MS +: patients with metabolic syndrome, MS -: patients without metabolic syndrome.

same individual of several metabolic abnormalities which predispose the occurrence of many cardiovascular complications (Vladimír et al., 2017). In this study, we assessed the association between uricemia and metabolic syndrome in our population.

Most patients were women (sex ratio = 0.54) with an average age of 56.63 years and extremes of 21 and 87 years. Similar results have been reported in many studies (Jeandel and Kouda Zeh, 1987; Siko, 1989; Wanvoegbe et al., 2017), which once again confirms that the prevalence of type 2 diabetes increases with age (Jeandel and Kouda Zeh, 1987; Siko 1989; Diouf et al., 2013).

The predominance of women is mainly linked to the high rate of physical inactivity of women in our society, which is a risk factor for obesity and cardiovascular disease (Bouzid et al., 2011). The mean duration of diabetes was 8.28 ± 6.54 years. This long duration, which indicates a prolonged evolution of the disease, exposes patients more to the occurrence of metabolic abnormalities. The frequency of metabolic syndrome in this study was 31%. Similar results have been reported in

Table 1. Comparison of variables between patients with hyperuricemia and patients with normal serum uric acid.

Variable	Hyperuricemia (n = 45)	Normouricemia (n = 108)	*p*
Age (years)	59.24±10.39	55.55± 11.36	0.062
Duration of diabetes (years)	9.12± 7.33	7.93± 6.18	0.44
BMI (kg/m2)	27.56± 5.39	25.00± 4.28	***0.013***
FPG (mmol/l)	8.37± 4.67	9.57± 2.12	0.083
HbA1c (%)	7.27± 1,47	8.54± 2.71	***0.013***
Microalbuminuria (mg/24h)	88.77± 259.48	37.02± 52.75	0.68
Urea (mmol/L)	5.32± 3.33	4.16± 1.33	***0.03***
Creatinine (μmol/L)	104.22± 44.99	86.45± 20.77	***0.046***
TC (mmol/L)	5.83± 1.37	5.59± 1.24	0.364
HDL-C (mmol/L)	1.63± 0.54	1.78± 0.52	0.05
TG (mmol/L)	2.41± 0.96	1.97± 1.29	***0.04***
LDL-C (mmol/L)	3.70± 1.24	3.36± 1.14	0.23

BMI: Body Mass Index, FPG: Fasting plasma glucose, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglyceride, LDL-C: low-density lipoprotein cholesterol.

Table 2. Correlations between acid uric levels and parameters of metabolic syndrome.

Variable	**r**	***p***
BMI (kg/m2)	0.12	0.13
FPG (mmol/l)	- 0.22	*0.005*
HbA1c (%)	- 0.25	*0.002*
Microalbuminuria (mg/24h)	0.19	*0.018*
TC (mmol/l)	0.10	0.23
HDL-C (mmol/l)	- 0.12	0.14
TG (mmol/l)	0.25	*0.002*
LDL-C (mmol/l)	0.12	0.13

r: correlation coefficient, BMI: Body Mass Index, FPG: Fasting plasma glucose, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglyceride, LDL-C: low-density lipoprotein cholesterol.

other studies where this frequency was 21, 24.3, 33.9, 34.7, 36.3 and 37% respectively in Saudi Arabia, Tunisia, in Iran, Turkey, Jordan and Palestine (El Bilbeisi et al., 2017). These variations, although close, are mainly explained by the differences in the criteria for defining the metabolic syndrome used across the different studies.

The WHO definition criteria used in this study was the best suited to the study population composed of type 2 diabetics. This relatively high frequency of metabolic syndrome could be linked to poor food hygiene as well as a high rate of physical inactivity, which are the main risk factors.

It found that 32% of patients with metabolic syndrome had hyperuricemia. Much higher frequencies have been found in other studies (Ismail et al., 2018). Hyperuricemia is frequently observed in diabetes as well as in metabolic syndrome and this is mainly linked to the increase in renal reabsorption of uric acid secondary to hyperinsulinemia (Quinones et al., 1995; Muscelli et al., 1996; Matsuura et al., 1998).

The study of the correlations between uricemia and the components of metabolic syndrome revealed a significant positive correlation with triglyceride level (*r = 0.25, p = 0.002*). The association between uricemia and triglyceridemia has also been demonstrated in other similar studies (Conen et al., 2004).

Indeed, it has been reported that the association between insulin resistance, hyperuricemia and hypertriglyceridemia is linked to a deficit in glyceraldehyde-3-phosphate dehydrogenase and to a loss of its sensitivity to insulin where the increase in triglycerides is due to an accumulation of glycerol-3-phosphate (Leyva et al., 1998).

We also found a significantly higher mean BMI value in patients with hyperuricemia (*p = 0.013*) although the weakly positive correlation found between uricemia and BMI was not significant (*r = 0.12; p = 0.13*). Likewise for total cholesterol level, a weak positive but not significant correlation was found (*r= 0.10; p = 0.23*). Indeed, several epidemiological and clinical studies have

shown a close correlation between hyperuricemia and obesity. In the study conducted by Masuo et al. it was shown that high concentrations of uric acid predispose to weight gain (Masuo et al., 2003).

It has also been suggested that hyperuricemia induces an alteration in the redox signaling pathways responsible for oxidative stress in adipocytes (Sautin et al., 2007) and this oxidative stress in adipose tissue is today recognized as being responsible for insulin resistance and cardiovascular disease. Finally, hyperuricemia can induce insulin resistance by causing vasodilation and an increase in blood flow, thus interfering with the action of nitric oxide which promotes glucose absorption (Khosla et al., 2005*).* It has also been suggested that hyperuricemia is linked to hyperinsulinemia by increased renal reabsorption of uric acid (Yoo et al., 2005; Lee et al., 2013). In contrast to these studies, the authors found negative correlation between fasting blood glucose and serum uric acid as well as glycated hemoglobin with respectively ($r = - 0.22$; $p = 0.005$) and ($r = - 0.25$; $p= 0.002$).

Conclusion

The association between hyperuricemia and metabolic syndrome has been demonstrated in many epidemiological studies and this hyperuricemia is considered by some authors to be a component of this syndrome. The authors have found a lower frequency of hyperuricemia in patients with metabolic syndrome than that reported in the literature.

Nevertheless, significant correlations have been highlighted between uricemia and some components of this syndrome, such as triglyceride level which is better correlated with serum uric acid concentrations compared to fasting blood sugar.

CONFLICTS OF INTERESTS

The authors have not declared any conflicts of interests.

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Study of isoforms of nicotinamide adenine dinucleotide phosphate oxidase of the heart in a model of rats fed on several vegetable oils

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Palm oil has long been incriminated in obesity, and this obesity would be responsible for the development of cardiac fibrosis, several authors have evoked the role of free radicals in the pathophysiological mechanism of the fibrotic response linked to obesity; the aim of this study was therefore to evaluate the profile of NOX2 in the development of cardiac fibrosis in rats subjected to a diet rich in the fat of several vegetable oils, in this case palm oil. A total of forty young male Wistar rats were subjected to several diets (soybean, red and branched palm oil, olive and lard). After twelve weeks of experimentation, the rats were sacrificed after anaesthesia, and the parameters of oxidative stress, inflammation and the level of interstitial fibrosis of the heart were assessed. Our study showed that red palm oil consumption did not lead to overexpression of oxidative stress parameters and inflammatory RNA markers. The expression of myocardial nicotine adenine dinucleotide phosphate oxidase did not change in rats consuming red palm oil compared to the control diet. However, consumption of palm olein, olive and lard resulted in a significant change in myocardial nicotine adenine dinucleotide phosphate oxidase activity. This study seems to show that red palm oil, because of its richness in antioxidants, would be less deleterious for the heart.

Key words: Oxidative stress-inflammation-palm oil-cardiac fibrosis.

INTRODUCTION

In recent years, obesity has become a matter of concern and is reportedly associated with metabolic disorders and Cardiovascular disease (CVD) (Stepien et al, 2012, 2014). Obesity leads to cardiac pressure overload and hypervolaemia (Kaltman and Goldring, 1976). Both factors lead to ventricular hypertrophy associated with

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increased collagen deposition (Xia et al., 2009; Ulasova et al., 2011) resulting in the development of cardiac fibrosis. Several molecular processes have been implicated in the regulation of the fibrotic response to obesity. These include activation of the renin-angiotensin-aldosterone system, oxidative stress, inflammation and leptin-induced actions (adipokines), (Eschalier et al., 2014). The mechanism induced by oxidative stress is not yet well understood. Reactive oxygen species (ROS) play an important role in the development of cardiovascular disease. In the cardiovascular system, several enzyme systems contribute to the formation of ROS. These include nicotinamide adenine dinucleotide phosphate oxidases (NOX), nitric oxide synthase, respiratory chain enzymes, cytochrome P450 monoxygenases and xanthine oxidase. Although all these systems are important in various disease states, NOX appears to play a central role in the dysfunction of these enzymes. The initial generation of ROS by NOX triggers the release of other sources of radical species (Landmesser et al., 2003). There are seven isoforms of NADPH oxidases expressed in mammals, but the most important for the cardiovascular system are NOX2, NOX1 and NOX4 (Lassegue and Clempus, 2003). NOX2 is the most widely expressed isoform. It is expressed in vascular smooth muscle cells, fibroblasts, endothelial cells and perivascular adipocytes (Van Buul et al., 2005; Infanger et al., 2006; Paravicini and Touyz, 2008). The expression profile of NOX varies in different disease states and their enzymatic activities can be increased in response to stimuli such as cytokines (De Keulenaer et al., 1998) and growth factors (Brandes et al., 2001). Palm oil (PA) has long been implicated as an important risk factor in the development of obesity and cardiovascular disease due to its high saturated fatty acid composition (Ellie Brown, 2005; Kabagambe et al., 2005). A diet containing myristic acid is thought to induce cell hypertrophy in the heart of C57BL / 6J mice (Russo et al., 2012).

According to some authors, palmitate induces apoptosis, activation of protein kinases associated with oxidative stress in ventricular cardiomyocytes (Miller et al., 2005). Studies on dietary fat composition remain one of the conflicting areas of biology due to the complexity of the structure and diversity of functions of FAs (Hamilton et al., 2001). The aim of this study was therefore to assess the profile of NOX2 in the development of cardiac fibrosis in rats fed a high-fat diet of several vegetable oils, in particular palm oil.

MATERIALS AND METHODS

Animal model and diets

A total of forty young male Wistar rats (Charles River, L'Arbresle, France) aged 6 weeks were used in the present study. The rats were housed, two per cage, under constant conditions of temperature (20-22°C), humidity (45-50%) and a standard dark cycle (20.00-08.00 h). Rats were randomly divided into five groups of eight animals and fed one of the following semi-purified diets for 12 weeks: (a) control diet containing 5% fat in the form of soybean oil (11% energy from fat) (Control), (b) high fat diets (55% energy from fat) rich in crude palm oil (cPO) (with 2.5% soybean oil and 30% cPO), (c) refined palm oil (rPO) diets (with 2.5% soybean oil and 30% rPO), (d) olive oil (OO) diets (with 2.5% soybean oil and 30% OO, (e) lard oil (LARD) diets (with 2.5% soybean oil and 30% LARD. The cPO and rPO were supplied by the company SANIA (Ivory Coast), the OO was purchased in a supermarket and the lard oil was supplied by the company CELYS, body fat food (ALVA, Rezé, France). The detailed composition of these experimental diets is given in Table 1. Rats were given free access to food and water throughout the experiment and body growth was determined weekly.

Sacrifice of rats and collection of samples

After twelve weeks of experimentation, the rats were sacrificed after anaesthesia by intraperitoneal injection of sodium pentobarbital (CevaSantéAnimale, Libourne, France). All animals were fasted the day before sacrifice. The blood, taken from the abdominal aorta, was divided into a heparinised tube and a dry tube. After centrifugation at 3000 g for 15 min at 4°C, the plasma and serum obtained were stored at -80°C. The red blood cells in the heparinised tube were rinsed twice with saline and stored at -80°C for SOD determination. For the isoprostane assay, plasma was frozen at -80°C with 0.005% BHT (3,5-di-tert-butyl-4-hydroxytoluene). The heart, after being rinsed with saline, was cut into two pieces. One piece for molecular biology stored at -80°C and one piece for histology in a 15 ml tube containing 10% formalin (Sigma, France) making five times the volume of the sample.

Oxidative stress parameters in blood

In plasma, apart from 15-F2t-isoprotane, oxidative stress parameters were determined spectrophotometrically. TBARS were determined by the method of Sunderman et al. (1985). Protein oxidation was assessed by measuring thiol groups (Faure and Lafond, 1995). Superoxide dismutase (SOD) activity was measured according to the method of Marklund (1976). The more specific parameter of lipid peroxidation, 15-F2t-isoprotane, was determined by mass spectrometry as described by Mas et al. (2008). Briefly, aliquots of plasma samples were spiked with 15-F2t-isoprostane D4 as an internal standard prior to extraction using an Agilent Bond Elut Certify II cartridge. Washes were performed with 50% methanol and ethyl acetate/hexane (1/3 v/v) and elution was performed with ethyl acetate/methanol (9/1 v/v). After esterification, the samples were analysed on a ThermoFinnigan Trace DSQ II instrument interfaced with a Trace GC Ultra 2000 gas chromatograph, equipped with an AS 3000 autosampler (ThermoFinnigan).

Expression of core mRNA

Total heart RNA was extracted with Trizol reagent (Invitrogen Life Technologies, Cergy Pontoise, France) according to the method of Chomczynski and Sacchi (1987) using a FastPrep-24 homogeniser (MP biomedicals, France). Reverse transcription reactions were performed on 500 ng of total RNA using a Takara reverse transcription kit (Takara Bio Europe, France) and RT-qPCR was performed using the LightCycler® 480 SYBR Green I Master (Roche Applied Science, France). Results were normalised to the RPLP0 gene. The genes studied were:

Superoxide dismutase 1 and 2 (SOD1 and SOD2); transglutaminase 2 (TGM2); Toll-like cell receptor (TLR2); soluble

Table 1. Composition of the study regimes.

Food (g/kg)	Control	red palm	Palm olein	Olive	Lard
Casein	**165**	**200**	**200**	**200**	**200**
Corn starch	442.5	233.8	233.8	233.8	233.8
Maltodextrin	144	80	80	80	80
Sucrose	100	53	53	53	53
Soybean oil	50	25	25	25	25
Oil red palm	0	300	**0**	**0**	**0**
Palm olein	**0**	**0**	300	**0**	**0**
Olive oil	**0**	**0**	**0**	300	**0**
Lard	**0**	**0**	**0**	**0**	300
Cellulose	50	50	50	50	50
Minerals (AIN-93M)	**35**	**42**	**42**	**42**	**42**
Vitamins (AIN-93M)*	**10**	**12**	**12**	**12**	**12**
L-Cystine	2	2.4	2.4	2.4	2.4
Choline chloride	1.5	1.8	1.8	1.8	1.8

interleukin 33 receptor (ST2); growth differentiation factor 15 (GDF15): transforming growth factor beta (TGFβ); interleukin 6 (IL6); metalloproteinase 2 (MMP2); NADPH Oxidase (NOX); collagen I (Col I) Supplementary Table 1.

Histological examinations

Sections of 5 µm were taken with a microtome (Leïca RM 2145, Microsystems Nussloch GmbH, Germany). Sirius red staining on 5 µm heart section slides was used to objectify areas of fibrosis on each category of rats using a microscope. Fibrosis was evaluated as the percentage of red stained pixels (collagenous tissue) in relation to the sum of green and red pixels (total tissue area) × 100% using Image J software. Immunostaining was performed using CD68 antibody (Bio-Rad, France) followed by infrared microscopy.

Statistical analyzes

The values were expressed as mean ± standard deviation. Statistical analysis is based on a two-way ANOVA, followed by Tukey Kramer's multiple comparison test. Statistical analyzes of the data were performed with StatView software (SAS Institute, Cary, NC, USA). The differences observed were considered significant for a p value <0.05. The Bravais-Pearson correlation test was used to evaluate linear regressions; the closer the values are to 1 (in absolute value), the stronger the relationship.

Ethical considerations

The research protocol for this study and all experimental procedures were approved by the local ethics committee in Montpellier, France (Reference CEEA-LR-12002).

RESULTS

Weight evolution kinetics of the animals

Figure 1 shows the kinetics of weight change of the rats fed the different diets. They were weighed weekly. The different diets resulted in a significant increase in the weight of the rats at the end of the 12 weeks compared to the rats fed the control diet.

Study of interstitial fibrosis and cardiomyocyte size

Figure 2A shows the micrographs of interstitial fibrosis lesions induced by the different oil-based diets in each category of rats on the heart (magnification x 200). Figure 2B shows the cardiomyocyte size in μm^2 and the proportion of interstitial fibrosis expressed as a percentage in animals fed the different diets. Cardiomyocyte size and the proportion of interstitial fibrosis were significantly increased in rats fed lard oil compared to the control diet. In contrast, they did not vary significantly in rats fed the red palm oil, palm olein diets compared to the control diet.

Oxidative stress, inflammatory and cardiac cytokine parameters

We determined oxidative stress parameters in the left ventricle of the hearts of rats fed the different diets by RTqPCR. RNA expression of the three NOX isoforms did not vary in the red palm oil fed rats compared to the control diet. The expression of NOX2 was significantly increased in rats fed lard oil (Figure 3). Furthermore, the RTqPCR study of SOD in the myocardium showed no variation between the different groups of rats (Figure 3). At the systemic level, no significant variation was observed in the oxidative stress parameters, regardless of the diet (Table 2). The expression of pro-inflammatory parameters (TGM2, TLR2 and ST2) did not vary in the red palm oil fed rats, but did vary in the other diets

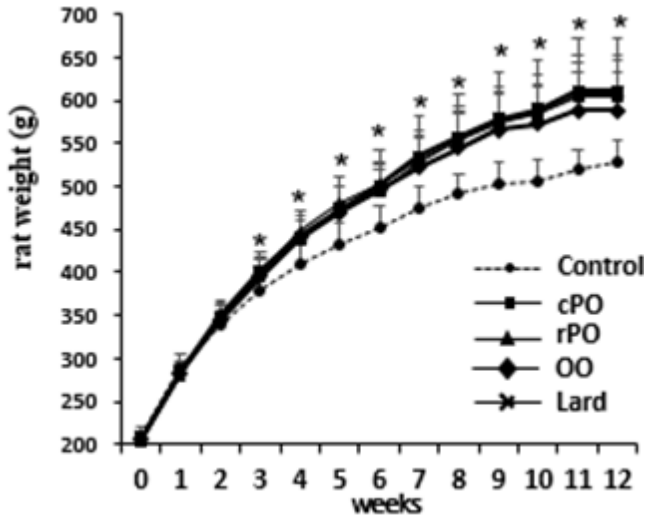

Figure 1. Kinetics of rat weights.

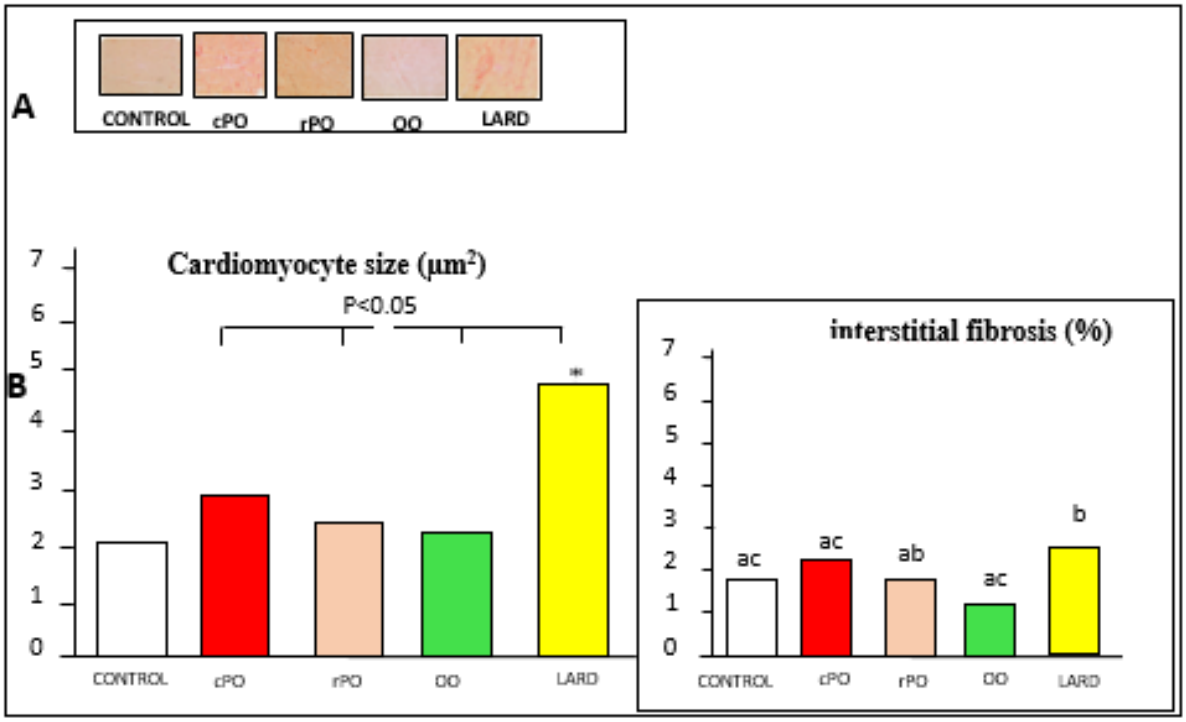

Figure 2. Effect of different regimens on cardiomyocyte size and proportion of interstitial fibrosis. The different letters (a, b, c) mean that the comparison between the different groups is statistically significant. The star * indicates the significant difference between the different groups compared to the control. Ctrl Diet = Control, Crude Palm Oil Diet = cPO, Refined Palm Oil Diet = rPO, Olive Oil Diet = OO, Lard Oil Diet = Lard.

(Figure 4).

Figure 5A shows micrographs of histological sections of the left ventricle (n = 10-20/rat) showing macrophage infiltration after labelling with primary CD68 antibody (magnification × 200). They show the effect of consumption of several vegetable oils on macrophage infiltration in the left ventricle. The results were expressed as a percentage of the tissue area infiltrated by macrophages. Results were expressed as mean values ± SD, n = 7-8 animals per group (Figure 5B). Our results show that macrophage

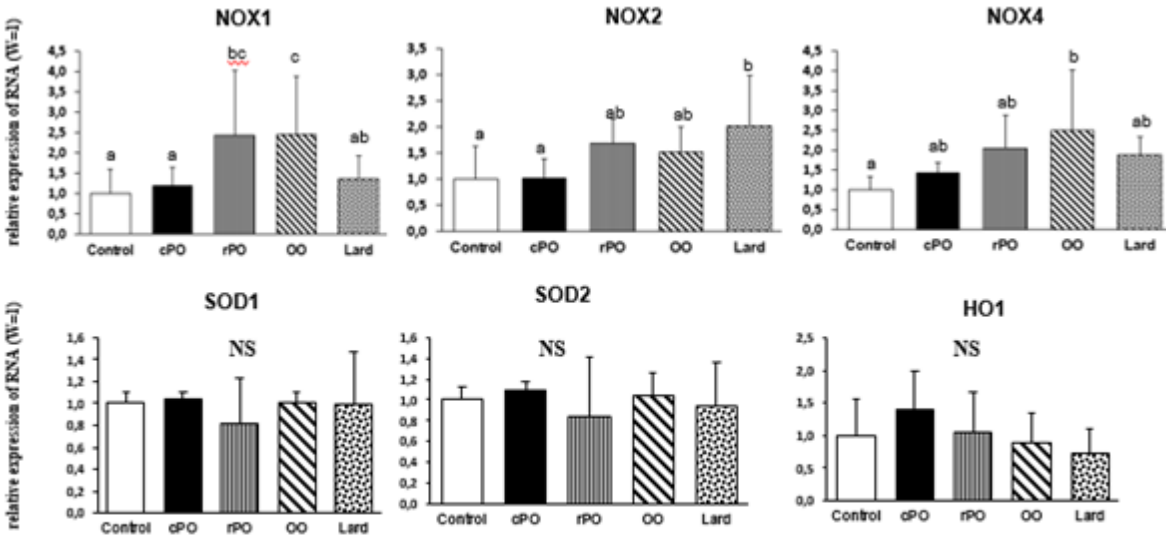

Figure 3. Study of markers of oxidative stress in the myocardium.

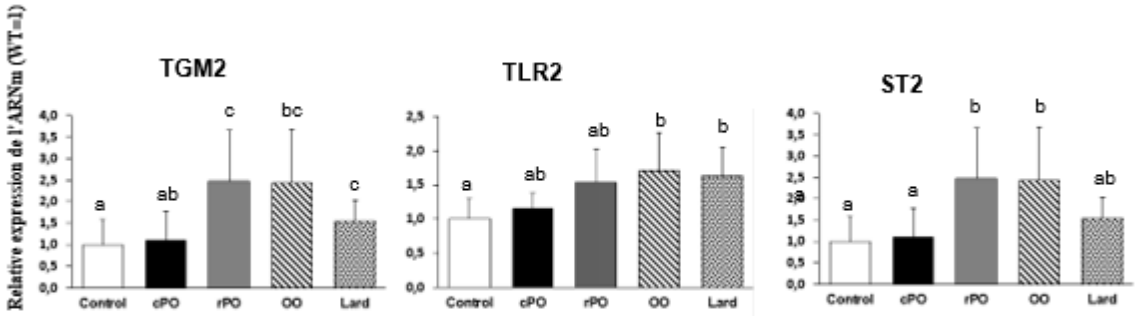

Figure 4. Studies of the parameters of inflammation and macrophage infiltration.

infiltration was significantly higher in the group of rats fed with lard oil. These macrophage infiltrations did not vary with red palm oil and the other diets. Also, the cardiac cytokines studied in the heart did not vary with the different diets (Table 3). On the other hand, a correlation between NOX2, NOX4 and the membrane receptor for interleukin 33 was observed in Figure 6. Results were expressed as mean ± standard deviation, n = 7–8 animals per group. Ctrl Diet = Control, Crude Palm Oil Diet = cPO, Refined Palm Oil Diet = rPO, Olive Oil Diet = OO, Lard Oil Diet = Lard.

DISCUSSION

We investigated the role of oxidative stress markers in the development of cardiac fibrosis in rats fed several high-fat diets, including crude palm oil, palm olein, olive and lard. Consumption of crude palm oil did not lead to overexpression of oxidative stress and inflammation parameters. Crude palm, palm olein and olive oil did not significantly increase the proportion of interstitial collagen and cardiomyocyte size compared to the control (lower calorie) diet.

Lard consumption resulted in a significant increase in cardiomyocyte size and proportion of interstitial fibrosis in rats at the end of 12 weeks compared to the control diet. Our study was in agreement with Kubant et al. (2015) who showed that lard fat consumption led to weight gain, visceral obesity, ventricular hypertrophy and cardiac fibrosis. High-fat diets induce obesity by altering carbohydrate metabolism (Lima-Leopoldo et al., 2011; White et al., 2013; Oliveira-Junior et al., 2014). The relative expression of NOX 1, NOX2 and NOX4 did not change in rats fed red palm oil compared to the control diet considered to be lower in calories. Consumption of red palm oil did not induce overexpression of NOX. Red palm oil appears to decrease free radical production through low NOX expression.

The nutritional benefits of palm oil in animals have

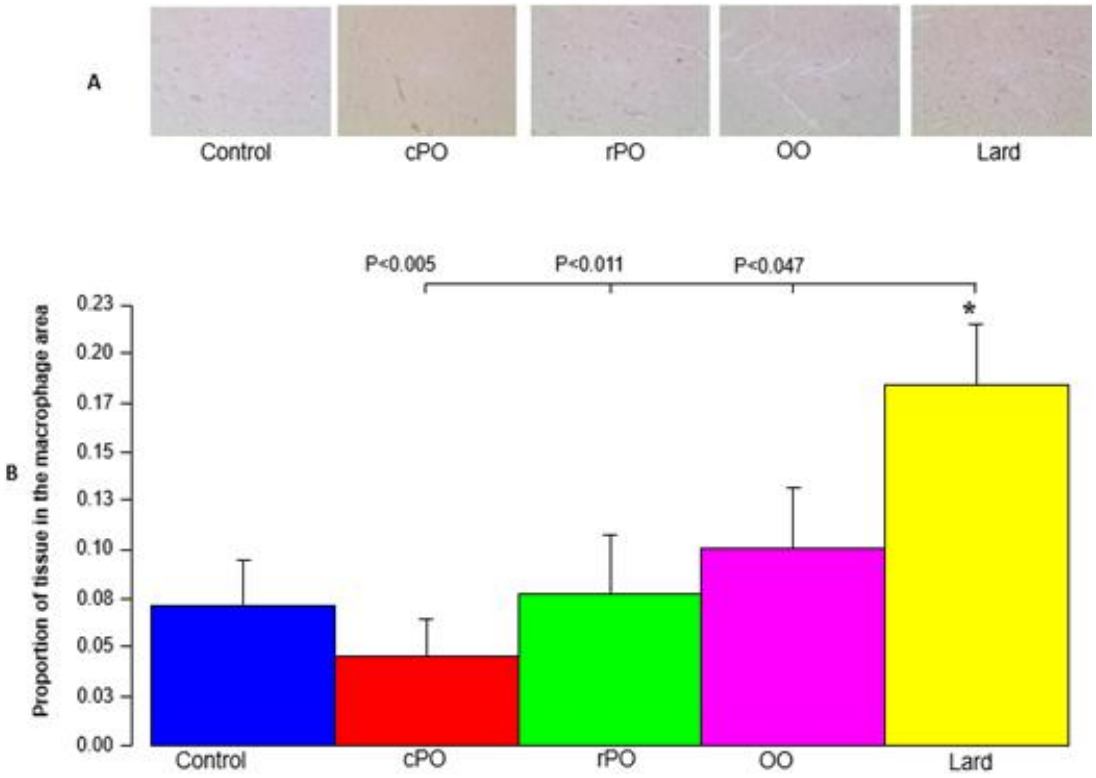

Figure 5. Effect of diets on macrophage infiltration.

Table 2: Study of plasma biomarkers of oxidative stress

Antioxidant system	Control	cPO	rPO	OO	Lard	P
SOD	324 ± 5,3	315 ± 8,7	296 ± 8,8	326 ± 6,6	311 ± 12	NS
GPx	14487±1170	16147±609	15945±1649	13927±478	13972±613	
Thiols (µmol/mL)	0,112±0,018	0,126±0,013	0,124±0,018	0,111±0,016	0,121±0,017	NS
TBARS(nmol/mL)	5,12 ±0,31	5,13 ±0,21	5,26 ±0,39	4,79 ±0,31	4,91 ±0,21	NS
15 -F_2t isoprostane (UA)	0,053±0,006	0,049±0,006	0,044±0,002	0,037±0,006	0,041±0,004	NS

The values of the parameters of the antioxidant system and of the products of lipid peroxidation are expressed as a mean ± SD (n = 7-8). SOD: Superoxide dismutase, GPx: Glutathione peroxidase, TBARS: Reactive substances of tiobarbituric acid. UA: arbitrary units, NOX: NADPH Oxidase, OH: Heme oxygenase, NS: means not significant. Ctrl Diet = Control, Crude Palm Oil Diet = cPO, Refined Palm Oil Diet = rPO, Olive Oil Diet = OO, Lard Oil Diet = Lard.

Table 3. Cardiac cytokine studies.

cytokines	Control	cPO	rPO	OO	LARD	P1(Hf/Cont)	P^2(Fat/Fat)
GDF 15	1,00±0.55	0.86±0.24	1.41±0.84	1.15±0.48	1.03±0.37	NS	NS
TGFβ	1,00±0.12	1.07±0.13	0.94±0.17	0.91±0.12	0.70±0.35	NS	NS
IL6	1,00±1.14	0.72±0.85	0.57±0.40	0.24±0.23	0.24±0.16	NS	NS
IL33	1,00±0.42	1.28±0.17	1.10±0.23	1.45±0.43	1.35±0.30	NS	NS
MCP1	1,00±0.23	0.98±0.44	1.78±1.53	1.09±0.73	1.09±0.61	NS	NS
COL1	1,00±0,25	1.08±0.27	1.17±0.17	0.93±0.35	1.00±0.28	NS	NS

TGM2: Transglutaminase2; TLR2: TOLL type cellular receptor; ST2: Iterleukin33 membrane receptor; GDF15: Growth differentiation factor-15. MMP2: Metalloproteinase 2; IL33: Interleukin 33; IL6: Interleukin 6; ColA1: Collagen A1: TGFβ: Transforming growth factor- β; NS: not significant, Ctrl Diet = Control, Crude Palm Oil Diet = cPO, Refined Palm Oil Diet = rPO, Olive Oil Diet = OO, Lard Oil Diet = Lard.

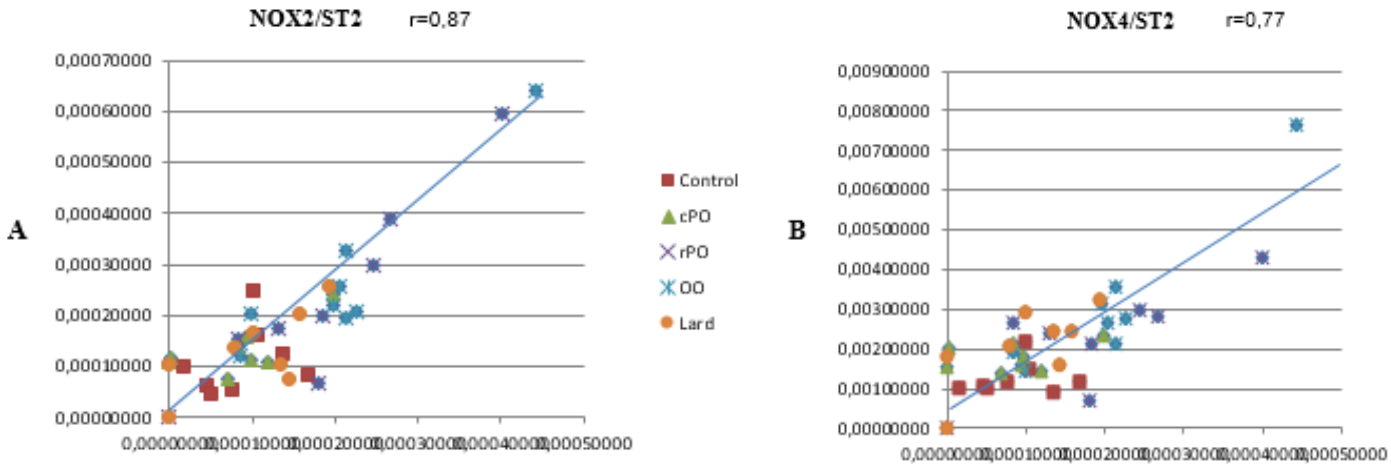

Figure 6. Correlation graph between NOX and ST2.NOX: NADPH Oxidase. ST2: Soluble interleukin 33 receptor, r: Bravais-Pearson correlation coefficient.

been elucidated by numerous studies (Suarna et al., 1993; Azlina et al., 2005) which have demonstrated the antioxidant effects of tocotrienols in palm oil. Coenzyme Q10 (ubiquinone), which is a natural coenzyme in palm oil, is a powerful free radical scavenger (Niklowitz et al., 2007). It has ten times the antioxidant power of carotenoids and vitamin E (Rosenfeldt et al., 2007). NOX2 is the most frequent isoform in the cardiovascular system (Infanger et al., 2006; Paravicini and Touyz, 2008). NOX2 was most expressed in rats consuming lard oil. This could explain the high proportion of interstitial fibrosis found in this group (Figure 2A and B).

Furthermore, NOX2 is involved in the recruitment of macrophages, which is an essential step in the formation of cardiac fibrosis (Van Buul et al., 2005). This observation is in agreement with the results of immunostaining with the CD68+ antibody, which shows a significant difference in macrophage infiltration in the cardiac cells of the lard-fed group of rats (Figure 5A and B). To further elucidate interstitial fibrosis, we analysed collagen I expression from myocardial tissue by RTqPCR. However, the collagen I RNA study did not vary significantly between oils.

Calligaris et al. (2013) confirmed fibrosis by overexpression of collagen types I and III in cardiac mRNA. In their model, cardiac remodelling was associated with thickening of cardiac fibres and the left ventricular wall, resulting in cardiac hypertrophy. NOX1 activity was increased in the palm olein and olive diets and NOX4 activity was higher in the olive group of rats. NOX1 and NOX4 although sharing 60 and 39% amino acid identity with NOX2 respectively (Guzik et al., 2004; Guzik et al., 2006) may have antagonistic functions (Schroder et al., 2012). Their physiological roles are poorly defined but they seem to play a central role in cell signalling (Arbiser et al., 2002; Cifuentes et al., 2006; Nauseef, 2008).

Nox4 and NOX1 mediate transforming growth factor β (TGF-β)-induced differentiation (Sturrock et al., 2006) In addition, cytokines have also been shown to regulate vascular NADPH oxidases, which associate inflammation with oxidative stress. In particular, tumour necrosis factor α (TNF-α) stimulates the expression and activation of Nox1, Nox2 and Nox4 in various vascular cells (Anilkumar et al., 2008; Basuroy et al., 2009; Moe et al., 2011). In our model, the different cytokines studied did not vary between regimes. Quantitative analysis of mRNA levels of molecules related to pro- or anti-inflammatory signals showed that TGFβ mRNA levels did not vary significantly between oils, contrary to data in the literature. (Okada et al., 2005; Lucas et al., 2010). To date, transforming growth factor beta (TGF-β) is the most potent and ubiquitous profibrogenic cytokine in fibrosis formation. It plays a central role in the development of fibrosis involving almost all organ systems (Lenz et al., 1996; Manouryet al., 2005; Rottoli et al., 2005). The factor growth differentiation factor-15 (GDF-15 is a member of the TGF-β superfamily, (Baan et al., 2015; Oshima et al.,2009).

In our study, GDF 15 expression did not vary significantly with diet. Our data are different from those of Tran et al. (2018) who objected that GDF15 deficiency promoted high-fat diet-induced obesity in knockout mice. On the other hand, pro-inflammatory parameters such as transglutaminase 2 (TGM2), Toll-like receptor (TLR2) and interleukin-33 membrane receptor (ST2) were significantly varied in the different diets, except in rats fed red palm oil (Figure 4A).There was a strong correlation between NOX4, NOX1 and soluble IL33 receptor. This observation could be explained by the fact that inflammation and oxidation are two fundamental processes underlying the pathogenesis of most human disease states.

Furthermore, it is now accepted that these two distinct mechanisms are in constant interaction, which is

particularly evident in the vessel wall (Lichtman et al., 2013; Miller et al., 2011; Takac et al., 2012). Vascular oxidative stress regulates the development of vascular inflammation which has recently been implicated in the pathogenesis of atherosclerosis (Harrison et al., 2011). Analysis of other parameters of the antioxidant system showed no significant difference between the different regimes, both in plasma and in the left ventricle.

In our study, the expression of superoxide dismutase 1 (SOD1) and superoxide dismutase 2 (SOD2) RNAs did not differ between the different diets. In contrast, in animal models, obesity was found to decrease the mRNA expression of antioxidant enzymes such as SOD, catalase (CAT) and GPx in white adipose tissue. (Furukawa et al., 2004). Several recent studies have shown that the expression of extracellular SOD or (SOD3) is decreased in the failing heart, and this has been associated with evidence of increased myocardial oxidative stress and endothelial dysfunction (Landmesser et al., 2003; Chen et al., 2005).

Noelia (2010) demonstrated that the absence of Gpx1 promotes angiotensin II-induced left ventricular hypertrophy and left heart dysfunction. The products of lipoperoxidation did not vary with the different diets, although all diets resulted in a significant increase in rat body weight compared to the control diet. Furthermore, F2 -IsoPs levels did not vary significantly between these diets, although multiple studies have clearly shown that F2 -IsoPs levels, measured in plasma, increase in adult obese patients (Basu, 2008; Kaikkonen et al., 2013). In the study by Furukawa et al. (2004), which involved several animal models of obesity, dietary fat intake caused an increase in lipid peroxidation (Furukawa et al., 2004).

Conclusion

Our study showed that red palm oil consumption did not result in overexpression of RNA parameters of oxidative stress and inflammatory markers. Myocardial NADPH oxidase expression did not change in rats consuming red palm oil compared to the control diet. However, consumption of palm olein, olive and lard resulted in a significant change in myocardial NADPH oxidase activity. This study seems to show that red palm oil, because of its high antioxidant content, is less harmful to the heart.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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SUPPLEMENTARY MATERIALS

Table 1. The gene sequences used in the study.

Primer sequence	Forward	Reverse
EMR1	GCCATAGCCACCTTCCTGTT	ATAGCGCAAGCTGTCTGGTT
GDF15	TGTTCCTGCTGCTCTTGCTG	TCGCACCTCTGGACTGAGTATC
COL A I	GACTGTCCCAACCCCCAAAA	TGGGTCCCTCGACTCCTATG
TGFβ	GACCGCAACAACGCAATCT	GACAGCCACTCAGGCGTATC
IL33	CCCTGAGCACATACAACGACC	CACCATCAGCTTCTTCCCATC
ST2	ATGATTGGCAAATGGAGAAT	TTCTAGACCCCAGGATGTTT
MCP1	TGTCTCAGCCAGATGCAGTT	CAGCCGACTCATTGGGATCA
SOD1	AGA GAG GCA TGT TGG AGA CCT G	ACG GCC AAT GAT GGA ATG CTC
SOD2	TCT GAA CGT CAC CGA GGA GAA G	AGT GCA GGC TGA AGA GCA AC
NOX1	CCAAACGTGACAGTGATGTATGC	AGCTGAAGTTACCATGAGAACCAA
NOX2	CGTATTGTGGGAGACTGGACTGA	AGGGCCCATCAACTGCTATCT
NOX4	GCCTAGGATTGTGTTTGAGCAGA	GCGAAGGTAAGCCAGGACTGT
TLR2	GAGGTCTCCAGGTCAAATCTCAG	ACACACCAGCAGCATCACAT
TGM2	CACTGTCAGCTACAACGG	CGCACCTTGATGAGGTTT
Rplpo	CACTGGCTGAAAAGGTCAAGG	GACTTGGTGTGAGGGGCTTA

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