Evaluation of Protein, Lipid and Inflammatory Profiles in Patients with Non-Hodgkin Lymphoma in the Western Region of Algeria

Meriem Rabia Zahzeh *, Touria Zahzeh
1 Laboratory of Molecular Microbiology, Proteomics and Health, University of Sidi Bel Abbes, Algeria

ABSTRACT

Introduction: Non-Hodgkin lymphoma (NHL) is part of the lympho-proliferative syndromes, it is a heterogeneous group of tumors whose incidence has been increasing in recent years. A disruption of protein and lipid profiles is verified during this pathology as well as an increase in the levels of specific markers of inflammation.

Objective: Our study aims to explore protein and lipid profiles and highlight an inflammatory syndrome via the assay of parameters of acute inflammation in patients with NHL and healthy subjects in western Algeria.

Methods: A case-control study comprising 100 patients with NHL and 40 healthy subjects was carried out. Protein and lipid profiles were respectively studied by assays for total protein and globulins and analysis of cholesterol and triglyceride levels. The inflammatory status was demonstrated by studying two parameters: C reactive protein (CRP) and sedimentation rate (VS).

Results: Our results demonstrated an increase in alpha1-globulin and a decrease in gamma-globulin in patients compared to controls (p <0.05). The lipid profile study showed no significant difference for cholesterol but a significant increase in triglycerides in patients with NHL (p <0.05). A highly significant difference in CRP and VS levels between our two groups (p <0.01) with a marked increase in patients demonstrated the presence of an inflammatory syndrome.

Conclusion: This study allowed us to highlight the alterations in protein and lipid profiles found in NHL and the presence of an inflammatory state, involved in the pathophysiology of non-Hodgkin lymphoma in particular.

Keywords: non-Hodgkin lymphoma, protein profile, lipid profile, CRP, VS.

INTRODUCTION

Non-Hodgkin lymphoma (NHL) is a cancer of cells of the immune system, in fact it is defined by clonal proliferation of B and T lymphocytes at different stages of differentiation and activation. As the immune system is ubiquitous, any organ can be the starting point of lymphoma1-2.

In 2015, NHL was the most common malignant hemopathy3. The annual rate standardized to the world population is 6.7 per 100,000 inhabitants per year, with an increase of 10 to 20% every 5 years. It occurs at any age, with half of the cases observed in subjects over 60 years and a third in over 75 years, with a male predominance and a sex ratio equal to 1.54.

In Algeria, more than 1,000 new cases are recorded each year, affecting 4% of the Algerian population. Extra-nodal non-Hodgkin lymphoma remains the most frequent with a histological prevalence of the large B-cell type, high grade and stage IV representing 60% of non-Hodgkin’s lymphomas5.

The pathogenesis of NHL remains poorly understood, nevertheless several factors are involved including some viruses, immunosuppression, genetic and environmental factors such as pesticides and smoking6.

Variations in the value of total protein levels and of some proteins including albumin, alpha 1 and 2 globulins, beta and gamma globulins can be indicative of some pathologies, in particular NHL.

The detection of an inflammatory syndrome found during a cancerous process is based on the measurement of the sedimentation rate (VS), associated with the assay of the reactive C protein which is a marker of choice among the proteins of the acute phase of inflammation7-8. Apart from inflammation, pathological variations in the CRP level are found in myocardial infarction, some cancers, immune diseases, chronic lymphoid leukemia, viral and bacterial infections and parasitic diseases9-10, 11.

A disruption of the lipid profile is observed in cancer patients, explained by the role of lipids in carcinogenesis, in fact, a positive correlation between the increase in
cholesterol and triglyceride levels and a higher risk of developing cancers has been reported by several studies\textsuperscript{12,13,14}. In order to make a contribution to any screening and treatment action for NHL, we explored the protein, inflammatory and lipid profiles in 100 patients with this pathology in a hospital environment, and at the same time in 40 healthy subjects representing the control group in the western region of Algeria.

**MATERIALS AND METHODS**

**Subjects and methods**

100 patients with NHL (52 men, 48 women; age 55.79 ± 1.48 years) admitted to the Hematology departments of the hospital-university centers of Temcen and Sidi Bel Abbes western of Algeria and 40 healthy subjects (23 men, 17 women; age 56.36 ± 3.01 years) were the subject of this study.

A questionnaire with different characteristics was submitted to the subjects and the consent of the participants was validated by their signatures. This work was approved by our local institutional ethics committee (CSF-SBA).

**Blood samples**

The samples were taken from the antecubital vein and collected in dry tubes for the assays of total proteins, CRP, cholesterol and triglycerides and in EDTA tubes for the assay of VS.

**Laboratory assays**

**Determination of total protein**

The protein assay method is based on the work of Skeggs and Hochstrasser (1964). The reaction is triggered by adding the Biuret reagent. During the reaction, proteins in the sample combine with copper in an alkaline medium to form a purple colored complex. The absorption of the complex is measured at the end point at 550 nm \textsuperscript{15}.

**Serum protein zone electrophoresis**

The HELENA method is based on the migration of serum proteins deposited on a "cellulose acetate" support subjected to an electric current according to their charge and their volume, the bands obtained form a proteinogram \textsuperscript{16}.

**C Reactive protein (CRP) assay**

The CRP-latex is a slide agglutination test for the qualitative and semi-quantitative detection of CRP in human serum. Latex particles coated with goat IgG anti-human CRP are agglutinated when mixed with samples containing CRP\textsuperscript{17}.

**Determination of sedimentation rate (VS)**

It is a test that involves allowing red blood cells to settle in a vertical tube. The distance traveled is measured for 1 hour. The reference method is the Westergreen method\textsuperscript{18}.

**Determination of cholesterol**

The method is based on the action of a cholesterol ester hydrolyase enzyme which will hydrolyze esters of cholesterol into free cholesterol and fatty acid. Free cholesterol is oxidized by cholesterol oxidase to cholesterol A and hydrogen peroxide which oxidizes the chromogen in the presence of peroxidase giving a red color. The quinone concentration (pink) is measured at 505 nm and proportional to the level of triglycerides\textsuperscript{20,21}.

**Statistical analyses**

Statistical analyses were performed using SPSS 22.0 software. Student’s t-test and Mann-Whitney U test used as appropriate allowed us to compare the two groups. Data were presented as values ± standard error of the mean. The p <0.05 and p <0.01 values were considered statistically significant and highly significant, respectively.

**RESULTS**

The characteristics of the patients and the controls are shown in Table I, no significant difference was observed between the two groups for age, sex and BMI (p > 0.05). B-cell NHL type was the most common (54%) followed by T-cell NHL (23%), with a predominance of stage I (35%).

| Variables          | Patients       | controls      | p      |
|--------------------|----------------|---------------|--------|
| Age (year)         | 55.79±1.48     | 56.36±3.01    | 0.823  |
| Sex (M/F)          | 52/48          | 23/17         | 0.521  |
| BMI (kg/m\textsuperscript{2}) | 22.3±0.34 | 22.36±0.63 | 0.912  |
| NHL type           |                |               |        |
| B-cell NHL         | 54             | -             | -      |
| T-cell NHL         | 23             | -             | -      |
| Manteau            | 8              | -             | -      |
| MALT               | 13             | -             | -      |
| NK-cell NHL        | 2              | -             | -      |
| Clinical stage     |                |               |        |
| CS I (%)           | 35             | -             | -      |
| CS II (%)          | 25             | -             | -      |
| CS III (%)         | 17             | -             | -      |
| CS IV (%)          | 23             | -             | -      |

p<0.05 was considered statistically significant. Data are presented as mean ± standard error. P: comparison between the two groups. BMI: body mass index, NHL: non Hodgkin lymphoma, CS: clinical stage.
Figure 1 represents the protein profile of patients with NHL and controls, no significant difference was noted for total protein, alpha2 and beta-globulin. An increase in alpha1-globulin and a decrease in gamma globulin were observed in patients compared to controls (p <0.05).

Figure 1: Protein profile of patients and controls

Regarding the lipid profile summarized in Figure II, our results show no significant difference for cholesterol but a significant increase in triglycerides in patients compared to controls (p <0.05).

Figure 2: Lipid profile of patients and controls

The dosage of inflammatory markers (Figure III) shows a very significant increase in CRP and VS in our patients (p <0.01).

Figure 3: Inflammatory markers of patients and controls

DISCUSSION

NHL is distinguished by the variation in serum protein levels. Our results show a significant increase in alpha 1 globulin in patients, which corresponds to an inflammatory syndrome. Many multiprotein plasma activation systems playing the role of mediators intervene during inflammation, one can cite the contact system where there are 4 proteins including factor XI. Activated, this protein stimulates the production of bradykinin which triggers the production by endothelial cells of PG2, thromboxane A2, tissue plasminogen activator causing pain, vasodilation, increased permeability vascular, and leukocyte margination. Alpha-1-globulin will block this factor and therefore participate in the regulation of the inflammatory response. Our results agree with those of the literature and with the high level of CRP found during our assays.

The gamma-globulin level, on the other hand, is markedly lower in the patients, with a significant difference between the two groups. The same observation was made by Balcells and by Gennes, who respectively associate hypogammaglobulinemia with a lymphoproliferative process and with lymphoid hemopathies. Thomas and Frenzel also note the decrease in this fraction during NHL.

The study of the inflammatory profile during our research has shown high levels for CRP and VS. The increase in CRP in patients with NHL is explained by the fact that neoplasias are pathologies with an inflammatory process. In fact, the tumor microenvironment, an essential player in the neoplastic process, promoting proliferation, survival and migration, is orchestrated by inflammatory cells. Inflammatory responses therefore play a decisive role at different stages of tumor development. An increase in inflammatory cytokine levels observed during inflammation leads to the transcripational induction of the CRP gene in hepatocytes of the liver.

CRP binds to stimulatory receptors, FcγRI and FcγRIIa, increasing phagocytosis and the release of inflammatory cytokines and pro-apoptotic cytokines inducing up regulation of p53 in monocytes and affecting their cell cycle kinetics which leads to the apoptosis by G2 / M arrest in some cancer cell lines. CRP also has the ability to induce phagocytosis of damaged cells. It also binds to the inhibitory receptor, FcγRIIb, blocking activation signals and playing an anti-inflammatory role.

Moreover, this protein appears to be not only a peripheral biomarker of inflammation and allows a referral towards effective immunotherapies, but also a useful and easy prognostic biomarker for NHL. The results of the study by Legouffe and al in 2009 indicate that 75% of patients with a CRP <10 mg / L survive 32 months after the diagnosis, while the group with a higher CRP level reaches a median survival of only 8.5 months.

Regarding the sedimentation rate, the higher it is, the greater the probability of being confronted with a systemic inflammatory disease. The studies by Brigden and Monti published in 1998 and 2013 respectively come to the same conclusion; a VS> 100 mm / hour has a positive predictive value of 90% for severe infectious, rheumatic or neoplastic disease. In patients known to have oncologic disease, VS greater than 100 mm / hour is often associated with the presence of metastases, although normal VS does not exclude it. In 2005, Durant et al also reported that tumor diseases are among the pathologies most frequently found during the exploration of an inflammatory syndrome.
objectified by an increase in VS⁴⁰. These conclusions are consistent with our findings. During carcinogenesis the lipid profile is also disturbed. Indeed, an induction of lipogenesis is essential for the proliferation of cancer cells.¹¹ The first tumor lipolytic factors (lipid mobilizing factor) were described in mice carrying lymphoma.¹² Lipids are important mediators of the immune response and inflammatory reactions.¹³ Our results demonstrate significantly elevated triglyceride values in our subjects compared to the controls which could be explained on the one hand by a decrease in the catabolism of TG with an increase in their half-life due to the decrease in the activity of LPL and LH.¹⁴ and on the other hand, by the fact that the environment of the tumor cell is characterized by an increase in the content of growth factors, which may be the cause of the over expression of the enzymes of lipogenesis in cancer cells. Alterations in the signaling pathways of these growth factors such as the MAPK pathway and the PI3K / AKT pathway are also responsible.¹⁵, ¹⁶ In addition, cancer cells are characterized by an increase in the expression of enzymes of lipogenesis inducing a high synthesis of lipids de novo (95%) despite a sufficient food intake, on the contrary, under physiological conditions the intake of lipids comes from the diet whereas de novo synthesis remains low except in the liver and adipose tissue which regulate lipid homeostasis.¹⁷, ¹⁸, ¹⁹

CONCLUSION

In conclusion, the study of protein and lipid profiles, as well as the determination of markers of inflammation in patients with non-Hodgkin lymphoma and healthy subjects, first demonstrated a pathological variation of some serum proteins and increased triglycerides. In addition, it demonstrated by the high levels of CRP and VS, the presence of an inflammatory state, both involved in the pathophysiology of cancers in general and of non-Hodgkin lymphoma in particular.

The unfavorable prognosis of patients and the problem of their therapeutic management could be linked to the alteration of protein and lipid metabolism but also to the chronic inflammation found during the neoplastic process.

Conflict of interest

The authors declare no conflicts of interest.

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