Infection, Genetics and Evolution 12 (2012) 1189–1194

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

The association between hepatitis C virus infection, genetic polymorphisms of oxidative stress genes and B-cell non-Hodgkin's lymphoma risk in Egypt

Hala Farawela [a], Mervat Khorshied [a], Iman Shaheen [a], Heba Gouda [a], Aya Nasef [a], Nelly Abulata [a], Hebat-Allah Mahmoud [c], Hamdy M. Zawam [b], Somaia M. Mousa [a,*]

[a] Department of Clinical and Chemical Pathology, Kasr Al-Aini School of Medicine, Cairo University, Cairo, Egypt
[b] Department of Medical Oncology, Kasr Al-Aini School of Medicine, Cairo University, Cairo, Egypt
[c] Egyptian Ministry of Health, Cairo, Egypt

ARTICLE INFO

Article history:
Received 19 January 2012
Received in revised form 4 April 2012
Accepted 5 April 2012
Available online 12 April 2012

Keywords:
HCV
Oxidative stress genes
Genetic polymorphism
Antioxidant enzymes
B-NHL
NHL risk

ABSTRACT

Hepatitis C virus (HCV) has been postulated to be an etiological agent for lymphoid malignancies. Polymorphisms in oxidative stress genes as; superoxide dismutase (SOD2), glutathione peroxidase (GPX1), catalase (CAT), myeloperoxidase (MPO) and nitric oxide synthase (NOS2) may influence non-Hodgkin's lymphoma (NHL) risk. HCV screening and polymorphisms in these five genes coding for antioxidant enzymes were studied in 100 Egyptian patients with B cell-NHL and 100 controls to clarify the association between HCV infection, oxidative stress genes polymorphisms and B cell-NHL risk. A significantly higher prevalence of HCV infection was detected among NHL patients relative to controls and this carried a 14-fold increased NHL risk (odds ratio (OR) = 14.3, 95% confidence interval (CI) = 5.4–38.3, $p < 0.0001$). GPX1 and MPO genetic polymorphisms conveyed increase in B-NHL risk (OR = 3.3, 95% CI = 1.4–7.4, $p = 0.004$ and OR = 4.4, 95% CI = 1.3–14.2, $p = 0.009$ respectively). Further analyses stratified by HCV infection revealed that concomitant HCV infection and GPX1 gene polymorphism had a synergetic effect on NHL risk with an OR of 15 (95%CI = 2.2–69.6, $p < 0.0001$). In addition, combined HCV infection and MPO gene polymorphisms had a pronounced NHL risk (OR = 9.2, 95%CI = 2.5–33.9, $p < 0.0001$). SOD2, CAT and NOS2 genetic polymorphisms were not found to confer increased NHL risk. This study revealed that HCV infection is a risk factor for NHL in Egypt. Polymorphisms in GPX1 and MPO genes may influence NHL risk in HCV infected Egyptian patients. Larger scale studies are warranted to establish this genetic susceptibility for NHL.

1. Introduction

Lymphoma genesis is a multifactorial process in which genetic, environmental, and infectious factors are involved. These factors may influence capacity of B-cells with pre-neoplastic changes to undergo transformation, or affect the quality of tumor-specific immune response (Skibola et al., 2007). Familial aggregation of non-Hodgkin's lymphoma (NHL) and the co-occurrence of NHL and other hematologic malignancies within families, provide evidence for genetic or common environmental etiologies for these conditions (Wang et al., 2007).

Chronic inflammation has been associated with a risk of NHL. Although the mechanisms underlying this association are uncertain, it is known that inflammation is associated with increased oxidative stress caused by the generation of reactive oxygen species (ROS) (Smedby et al., 2006). It propagates pro-inflammatory cytokines including interleukin-1 that stimulates B-lymphocytes to produce antibodies responsible for initiation of B-cell activation. ROS normally exist in a physiologic harmony with cellular antioxidants. Oxidative stress occurs when this critical balance is disrupted or compromised (McElnea et al., 2011).

Cells are protected from the harmful effects of ROS by antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX1), catalase (CAT), nitric oxide synthase (NOS2) and myeloperoxidase (MPO) (Betteridge, 2000). These and other polymorphisms that impair anti-oxidative capacity may influence NHL risk by increasing levels of pro-inflammatory cytokines leading to increased B-cell activation (Lightfoot et al., 2006).

A number of polymorphisms in the genes coding for antioxidant enzymes have been identified. T–C transition in codon 16 of the gene coding for SOD2 results in a valine to alanine substitution. This substitution has been associated with increased risk of cancer (Liu et al., 2004; Olson et al., 2004; Wang et al., 2001). Proline (Pro) to leucine (Leu) substitution of GPX1 has been associated with lung cancer (Ratnasinghe et al., 2000), breast cancer (Ravn-Haren et al.,

* Corresponding author. Address: Department of Clinical and Chemical Pathology, Kasr Al-Aini School of Medicine, Cairo University, P.O. Box 99, Manial El-Roda, Cairo 11553, Egypt. Tel.: +20 111 8942138; fax: +20 2 23654480.
E-mail address: smamousa@gmail.com (S.M. Mousa).

1567-1348/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.meegid.2012.04.007

2006) and cardiovascular disease (Hamanishi et al., 2004). The C to T polymorphisms in codon −262 in the promoter region of CAT gene potentially lead to variations in cellular enzyme activities. CAT-C262T polymorphism has been associated with an increased risk of breast (Ahn et al., 2005) and pancreatic cancer (Cullen et al., 2003).

MPO is a lysosomal enzyme important in neutrophils and monocytes that produces a potent oxidant, hypochlorous acid as well as other ROS, which have antimicrobial activity. The role of MPO-G463A genetic polymorphism in cancer susceptibility is controversial. The low activity MPO-463A allele has been found associated with decreased risk for a number of tumors (Hung et al., 2004: Olson et al., 2004), while the high activity allele (G) has been associated with increased risk for acute lymphoblastic leukemia (Krajinovic et al., 2002). For gene coding for iNOS enzyme (NOS2A), serine (Ser) to leucine (Leu) substitution at codon 608 (Ser608Leu) has been reported to confer higher enzymatic activity (Shen et al., 2004; Stuehr, 1999). High levels of iNOS expression as well as nitric oxide (NO) have been demonstrated in human B-NHL (Mendes et al., 2001).

Hepatitis C virus (HCV) has been postulated to be an etiological agent for some types of lymphoid malignancies. However, the etiologic fraction of NHL attributable to HCV varies greatly by country (Dal Maso and Franceschi, 2006). Whereas a high prevalence of HCV infection in NHL patients has been shown to exist in geographical areas of high HCV prevalence (Cowgill et al., 2004), studies in many other areas have not established any form of association (Varma et al., 2011). Research in this field has progressed significantly over the last decade as the number of patients diagnosed with HCV and B-NHL is rising incrementally. It is therefore becoming crucial to fully understand the pathobiologic link of HCV in B-cell lymphoma genesis.

The aim of the current study was to assess the link between genetic polymorphisms in five oxidative stress genes (SOD2, GPX1, CAT, MPO and NOS2A), HCV infection and B-NHL risk in Egypt. To our knowledge, this is the first study that correlates the effect of oxidative stress multi-gene polymorphisms as well as HCV infection to B-NHL risk.

2. Patients and methods

2.1. Study population

The current study was carried out on 100 newly diagnosed Egyptian patients with B-cell NHL. Patients were chosen during the period between March 2010 and April 2011 among cases referred to the Department of Medical Oncology, Kasr Al-Aini Hospital, Faculty of Medicine, Cairo University, after taking their informed consents. Hundred unrelated matched healthy Egyptian blood donors were included in the current study as a control group. The research protocol was approved by the research Ethics committee of the Departments of Clinical pathology and Medical Oncology, Cairo University. Diagnosis of B-NHL was based on lymph node excision biopsy from the affected group of lymph nodes. Bone marrow biopsy was done for staging. Histopathological and immunohistochemical studies were done to confirm the diagnosis and for proper sub-typing according to the WHO classification (Swerdlow et al., 2008).

The extent of the disease was categorized according to the Ann Arbor classification and the performance status was assessed using the Eastern Cooperative Oncology Group (ECOG) criteria (Armitage, 2005).

2.2. Genotyping of the candidate genes

Genomic DNA was extracted from whole blood using QIAamp DNA Blood Mini kit (Qiagen, USA). Genotyping of the genes was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. Reagents and primers were provided by Qiagen, USA. The primer sequences and amplification conditions are shown in Table 1. The PCR amplified products were digested using specific restriction endonuclease for each gene (New England Biolabs, USA). The restricted PCR products were electrophoresed through 2% ethidium bromide stained agarose gel, and visualized by ultraviolet light. The restriction enzymes used and suspected length of the restriction fragments are shown in Table 2. For quality control, genotyping of 10% of the samples was repeated. Samples were randomly chosen and interpreted blindly by two different observers. The results obtained were identical to the initial results.

2.3. Detection of HCV RNA

Patients and controls' sera were tested for HCV RNA using RT-PCR method.

2.4. Statistical methods

Data was analyzed using SPSS win statistical package version 17. Hardy–Weinberg equilibrium for genotype frequencies was assessed in controls using Chi-square test. Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann–Whitney test (non-parametric *t*-test). Odds ratio (OR) with its 95% confidence interval (CI) were used for risk estimation. A p-value <0.05 was considered significant.

Table 1
Primer sequences and PCR conditions for different polymorphisms.

Polymorphism	Sequence (5′ → 3′)	PCR program	Ref.
SOD2 (Val16Ala)	F:GCTGTGCTTTCTCGTCTTCAG R:TGGTACTTCTCCTCGGTGACG	38× (30″94 °C + 30″60 °C + 30″72 °C)	Nemoto et al. (2007)
GPX1 (Pro197Leu)	F:TTATGACCGACCCCAAGCTCA R:ACAGCAGCACTGCAACTGCC	35× (1′94 °C + 1′56 °C + 1′72 °C) → 4′72 °C	Nemoto et al. (2007)
CAT (C-262T)	F:AATCAGAAGGCAGTCCTCCC R:TCGGGGAGCACAGAGTGTAC	35× (30″94 °C + 30″63 °C + 30″72 °C) → 5′72 °C	Nemoto et al. (2007)
MPO (G-463A)	F:CCGTATAGGCACACAATGGTGAG R:GCAATGGTTCAAGCGATTCTTC	5′90 °C → 35× (1′94 °C + 1′56 °C + 1′72 °C) → 7′72 °C	Rajp et al. (2007)
NOS2 (Ser608Leu)	F:CATATGTATGGGAATACTGTATTTCAG R:TCTGAACTAGTCACTTGAGG	40× (30″94 °C + 1′60 °C + 1′72 °C)	van de Sande et al. (2007)

Table 2
Restriction enzymes and length of the restriction fragments for different polymorphisms.

Polymorphism	Restriction enzyme	Allele	Length (bp)	Ref.
SOD2 (Val16Ala)	BsaWI	Wild (V/V) Homotype (A/A) Heterotype (V/A)	267 183 + 84 267 + 183 + 84	Flekac et al. (2008)
GPX1 Pro197Leu)	HaeIII	Wild (P/P) Homotype (L/L) Heterotype (P/L)	88 + 82 + 60 148 + 82 148 + 88 + 82 + 60	Forsberg et al. (1999)
CAT (C-262T)	HinfI	Wild (C/C) Homotype (T/T) Heterotype (C/T)	250 177 + 73 250 + 177 + 73	Park et al. (2006)
MPO (G-463A)	AciI	Wild (G/G) Homotype (A/A) Heterotype (G/A)	168 + 121 + 61 289 + 61 289 + 168 + 121 + 61	Cascorbi et al. (2000)
NOS2 (Ser608Leu)	BsaI	Wild (Ser/Ser) Homotype (Leu/Leu) Heterotype (Ser/Leu)	680 490 + 190 680 + 490 + 190	van de Sande et al. (2007)

3. Results

Demographic data of NHL patients are presented in Table 3. No significant correlation could be detected between different genotype variants and clinical stage of the disease. The frequencies of HCV infection as well as different gene polymorphisms among patients and control groups are shown in Table 4. Genotype frequencies were in Hardy–Weinberg equilibrium. HCV screening revealed HCV positivity in sera of 43% of NHL patients and 5% of the control group. This carried an almost 14-fold increased NHL risk with HCV infection (OR = 14.3, 95% CI = 5.4–38.3, $p < 0.0001$). Comparing the frequency of different genotypes among patients and controls (Table 5) revealed that: Individuals homozygous for GPX1-Pro197Leu gene mutation (L/L homotype) had an increased risk of B-NHL (OR = 3.3, 95% CI = 1.4–7.4, $p = 0.004$). This risk increase was not observed with P/L genotype (P/L compared to the referent P/P).

Evaluation of MPOG-463A gene mutations (G/A or A/A compared to the referent G/G) revealed that homozygous mutation of MPO-G463A gene conferred increased NHL risk (OR = 4.4, 95% CI = 1.3–14.2, $p = 0.009$). No statistically significant difference could be detected in the distribution of NOS2-Ser608Leu, SOD2-Val16Ala and CAT-C262T genotypes between B-NHL patients and controls.

Further analyses stratified by HCV infection revealed that concomitant HCV infection and GPX1-Pro197Leu gene mutation had a synergetic effect on NHL risk (OR = 15, 95%CI = 2.2–69.6, $p < 0.0001$). In addition, HCV infected patients carrying MPOG-463A gene mutations had a pronounced risk to develop NHL (OR = 9.2, 95%CI = 2.5–33.9, $p < 0.0001$).

4. Discussion

To date, our study offers the first analysis of the interaction between HCV infection, polymorphisms in oxidative stress genes and B-cell NHL risk. We detected about three fold-increased NHL risk with GPX1 L/L homotypes. A modest increase in the risk for NHL was previously reported with GPX1 mutant genotypes (Lightfoot et al., 2006), and had been attributed to reduction in glutathione peroxidase activity that had been associated with poor prognosis in diffuse large B-cell lymphomas (Tome et al., 2005), suggesting a possible role for this enzyme in the etiology and prognosis of NHL.

In the current study, MPO A/A homotype was found to be significantly higher in NHL patients compared to controls, and conferred an almost four fold increased risk for NHL. On the contrary, in the study of Wang et al., 2006, GA heterozygote was associated with increased NHL risk but not the AA homozygote. This inconsistency in the literature may reflect a potential gene–environmental interaction. In a recent meta-analysis, the protective effect of the heterotype G/A against cancer was found to be present in Europeans but not in Asians reflecting the importance of ethnic variations (Chu et al., 2010). Only one reported study investigated MPO-G463A polymorphism and risk of lung cancer in an African population and observed no significant associations (London et al., 1997).

Table 3
Characteristics of NHL patients.

Characteristic	NHL patients ($n = 100$) n (%)
Age (years)	18–75
Sex	
Male	63 (63)
Female	37 (37)
B symptoms	28 (28)
Lymphadenopathy	59 (59)
Lymph nodes involved	
Cervical	39 (66.1)
Axillary	26 (44.1)
Inguinal	25 (42.4)
Submandibular	17 (28.8)
Abdominal	12 (20.3)
Para-aortic	8 (13.6)
Mesenteric	2 (3.4)
Extranodal involvement	73 (73)
Splenomegaly	32 (32)
Hepatomegaly	26 (26)
Clinical stage	
IA	38 (38)
IB	3 (3)
IIA	17 (17)
IIB	2 (2)
IIIA	28 (28)
IIIB	6 (6)
IVA	3 (3)
IVB	3 (3)
Case pathology	
DLBCL[a]	76 (76)
SLL	8 (8)
Burkitt	7 (7)
Follicular	5 (5)
Marginal zone[b]	4 (4)

DLBCL = diffuse large B cell lymphoma, SLL = small lymphocytic lymphoma.
[a] Includes cases with T-cell rich B cell lymphoma ($n = 6$).
[b] Includes one case of MALT lymphoma.

Table 4
Distribution of HCV, *SOD2*, *GPX1*, *CAT*, *MPO* and *NOS2* genotypes among NHL patients and control groups.

Gene	Control (n = 100)		NHL (n = 100)	
	HCV positive, n = 5	HCV negative, n = 95	HCV positive, n = 43	HCV negative, n = 57
SOD2 (Val16Ala)				
Wild genotype	1 (1%)	11 (11%)	4 (4%)	6 (6%)
Mutant genotypes	4 (4%)	84 (84%)	39 (39%)	51 (51%)
GPX1 (Pro197Leu)				
Wild genotype	3 (3%)	65 (65%)	18 (18%)	32 (32%)
Mutant genotypes	2 (2%)	30 (30%)	25 (25%)	25 (25%)
CAT (C-262T)				
Wild genotype	0 (0%)	23 (23%)	11 (11%)	15 (15%)
Mutant genotypes	5 (5%)	72 (72%)	32 (32%)	42 (42%)
MPO (G-463A)				
Wild genotype	2 (2%)	64 (64%)	19 (19%)	30 (30%)
Mutant genotypes	3 (3%)	31 (31%)	24 (24%)	27 (27%)
NOS2 (Ser608Leu)				
Wild genotype	4 (4%)	64 (64%)	28 (28%)	36 (36%)
Mutant genotypes	1 (1%)	31 (31%)	15 (15%)	21(21%)

Table 5
Comparison between the frequency of *SOD2*, *GPX1*, *CAT*, *MPO* and *NOS2* genotypes among NHL patients and control groups.

Gene	Genotype	Control (N = 100) n (%)	NHL (N = 100) n (%)	OR (95%CI)	p Value
SOD2 (Val16Ala)	V/V	12 (12)	10 (10)	1 (ref)	–
	V/A	49 (49)	50 (50)	1.2 (0.5–3.1)	0.7
	A/A	39 (39)	40 (40)	1.2 (0.5–3.2)	0.7
GPX1 (Pro197Leu)	P/P	68 (68)	50 (50)	1 (ref)	–
	P/L	22 (22)	26 (26)	1.6 (0.8–32)	0.2
	L/L	10 (10)	24 (24)	**3.3 (1.4–7.4)**	**0.004**
CAT (C-262T)	C/C	28 (28)	26 (26)	1 (ref)	–
	C/T	53 (53)	49 (49)	1 (0.5–1.9)	1
	T/T	19 (19)	25 (25)	1.4 (0.6–3.2)	0.4
MPO (G-463A)	G/G	66 (66)	49 (49)	1 (ref)	–
	G/A	30 (30)	38 (38)	1.7 (0.9–3.1)	0.08
	A/A	4 (4)	13 (13)	**4.4 (1.3–14.2)**	**0.009**
NOS2 (Ser608Leu)	Ser/Ser	68 (68)	64 (64)	1 (ref)	–
	Ser/Leu	30 (30)	30 (30)	1.1 (0.6–2.0)	0.8
	Leu/Leu	2 (2)	6 (6)	3.2 (0.6–16.4)	0.3

Boldface indicates statistically significant results.

Thus, larger scale studies are warranted to further validate ethnic differences in the effect of this polymorphism on cancer risk.

We could not detect significant increased NHL risk in patients carrying NOS2 gene mutations. Comparing our findings with previous reports revealed that Wang et al., 2006 detected a doubling of NHL risk, while Lan et al., 2007 found no association between genetic polymorphisms in NOS2 gene and susceptibility to NHL. NOS2 homozygous mutation has been reported to confer higher enzymatic activity and iNOS expression (Shen et al., 2004; Stuehr, 1999), resulting in increased NO production, a free radical whose role in tumor biology is still controversial (Förstermann et al., 1995). On one hand, NO can favor tumor growth and development by stimulating angiogenesis (Gallo et al., 1998) and causing immunosuppression (Mannick et al., 1994); on the other hand, NO could play a role in tumor regression through its ability to induce apoptosis (Atik et al., 2006) and to facilitate an immune rejection of the tumor (Hu et al., 2004).

Genotype distributions of SOD2-Val16Ala and CAT-C262T in NHL patients were similar to that among controls. Therefore, they did not confer increase risk of NHL as concomitant with previously published data (Lightfoot et al., 2006; Wang et al., 2006).

In a recent study, no significant association was detected between CAT-C262T polymorphism and the risk of any type of skin cancer but the risk was significantly modified by history of severe sunburns and dietary intake of carotenoids (He et al., 2010). This may be another example of gene–environment interaction that needs to be further investigated in cases of NHL.

HCV prevalence in NHL patients varies in different areas of the world. Most of the published data are retrospective paving the way for the argument that the association between HCV and NHL may be related to the increased risk that exists for NHL patients to be infected by the virus during therapy and hospitalization than the viral infection leads to lymphoma genesis (Varma et al., 2011).

We observed a significant higher prevalence of HCV infection among newly diagnosed NHL patients prior to therapy relative to controls and this conferred a 14-fold increased NHL risk with HCV infection. This is in accordance with that previously reported in several Egyptian studies (Abdel-Fattah and Yassine, 2007; Cowgill et al., 2004). However, this fold increase was higher than that previously reported in Egypt (Cowgill et al., 2004) and worldwide (de Sanjose et al., 2008). This may be explained by lower prevalence of HCV infection among our healthy controls (5%) compared to previous reports in Egypt (14.7% and 27%) (El-Zanaty and Way, 2009; Goldman et al., 2009). The lower prevalence of HCV infection in our control group may be attributed to relatively small sample size that did not represent the whole population and the strict exclusion criteria conducted for controls selection being candidates for blood donation.

Various studies showed that there is a great association of HCV with B-cell NHL in countries like Italy, Brazil, Spain, Saudi Arabia, Israel, Yemen, Spain, Japan and some parts of the United States. However, there are studies which have shown negative association as in Canada, Turkey, and the UK. Many other studies have not established any form of association as in Greece, Mexico, France, Thailand, The Netherlands and Korea (Varma et al., 2011).

In the current study, concomitant HCV infection modified NHL risk carried by oxidative stress genes polymorphisms. HCV-infected patients carrying mutant alleles of GPX1 and MPO genes had an increased NHL risk (9-fold increased risk with GPX1 and 15-fold increased risk with MPO genetic mutations). These results should be confirmed in future studies including larger number of HCV positive controls before considering genetic polymorphism of oxidative stress genes an additional factor increasing the risk of NHL in HCV positive Egyptians. In similar studies, HCV-infected Moroccan patients carrying CAT T/T genotype (Ezzikouri et al., 2010) and SOD A/A genotype (Ezzikouri et al., 2008) were found to have a higher risk to develop hepatocellular carcinoma when compared with controls.

The present study could have yielded more consistent results if included more participants, and if the treatment regimen given for NHL and HCV, together with the response to therapy were available.

In conclusion, our study provides an evidence for the possible association between chronic HCV infection, genetic variations in GPX1 and MPO and B-cell NHL risk. Furthermore, NOS2A-Ser608-Leu, SOD2-Val16Ala and CAT-C262T are not found to be genetic predisposing factors for the development of B-cell NHL in Egypt. These findings provide additional clues to the etiology of NHL and support identifying additional genes and environmental exposures that may modulate oxidative stress with the ultimate goal of identifying novel prevention approaches.

References

Abdel-Fattah, M.M., Yassine, O.G., 2007. Non-Hodgkin's lymphomas in Alexandria, Egypt; incidence rates and trend study (1995–2004). Eur. J. Cancer Prev. 16 (5), 479–485.

Ahn, J., Gammon, M.D., Santella, R.M., Gaudet, M.M., Britton, J.A., Teitelbaum, S.L., Terry, M.B., Nowell, S., Davis, W., Garza, C., Neugut, A.I., Ambrosone, C.B., 2005. Associations between breast cancer risk and the catalase genotype, fruit and vegetable consumption, and supplement use. Am. J. Epidemiol. 162(10), 943–952.

Armitage, J.O., 2005. Staging non-Hodgkin lymphoma. CA Cancer J. Clin. 55, 368–376.

Atik, E., Ergin, M., Erdoğan, S., Tuncer, I., 2006. Inducible nitric oxide synthase and apoptosis in human B cell lymphomas. Mol. Cell. Biochem. 290 (1–2), 205–209.

Betteridge, D.J., 2000. What is oxidative stress? Metabolism 49 (Suppl. 1), 3–8.

Cascorbi, I., Henning, S., Brockmöller, J., Gephart, J., Meisel, C., Müller, J.M., Loddenkemper, R., Roots, I., 2000. Substantially reduced risk of cancer of the aerodigestive tract in subjects with variant −463A of the myeloperoxidase gene. Cancer Res. 60(3), 644–649.

Chu, H., Wang, M., Wang, M., Gu, D., Wu, D., Zhang, Z., Tang, J., Zhang, Z., 2010. The MPO-463G > A polymorphism and cancer risk: a meta-analysis based on 43 case-control studies. Mutagenesis 25 (4), 389–395.

Cowgill, K.D., Loffredo, C.A., Eissa, S.A., Mokhtar, N., Abdel-Hamid, M., Fahmy, A., Strickland, G.T., 2004. Case-control study of non-Hodgkin's lymphoma and hepatitis C virus infection in Egypt. Int. J. Epidemiol. 33 (5), 1034–1039.

Cullen, J.J., Mitros, F.A., Oberley, L.W., 2003. Expression of antioxidant enzymes in diseases of the human pancreas: another link between chronic pancreatitis and pancreatic cancer. Pancreas 26 (1), 23–27.

Dal Maso, L., Franceschi, S., 2006. Hepatitis C virus and risk of lymphoma and other lymphoid neoplasms: a meta-analysis of epidemiologic studies. Cancer Epidemiol. Biomark. Prev. 15 (11), 2078–2085.

de Sanjose, S., Benavente, Y., Vajdic, C.M., Engels, E.A., Morton, L.M., Bracci, P.M., Spinelli, J.J., Zheng, T., Zhang, Y., Franceschi, S., Talamini, R., Holly, E.A., Grulich, A.E., Cerhan, J.R., Hartge, P., Cozen, W., Boffetta, P., Brennan, P., Maynadié, M., Cocco, P., Bosch, R., Foretova, L., Staines, A., Becker, N., Nieters, A., 2008. Hepatitis C and non-Hodgkin lymphoma among 4784 cases and 6269 controls from the International Lymphoma Epidemiology Consortium. Clin. Gastroenterol. Hepatol. 6 (4), 451–458.

El-Zanaty, F., Way, A., 2009. Egypt Demographic and Health Survey 2008. Egyptian: Ministry of Health (El-Zanaty and Associates and Macro International, Cairo), pp. 252.

Ezzikouri, S., El Feydi, A.E., Chafik, A., Afifi, R., El Kihal, L., Benazzouz, M., Hassar, M., Pineau, P., Benjelloun, S., 2008. Genetic polymorphism in the manganese superoxide dismutase gene is associated with an increased risk for hepatocellular carcinoma in HCV-infected Moroccan patients. Mutat. Res. 649(1–2), 1–6.

Ezzikouri, S., El Feydi, A.E., Afifi, R., Benazzouz, M., Hassar, M., Pineau, P., Benjelloun, S., 2010. Polymorphisms in antioxidant defence genes and susceptibility to hepatocellular carcinoma in a Moroccan population. Free Radic. Res. 44 (2), 208–216.

Flekac, M., Skrha, J., Hilgertova, J., Lacinova, Z., Jarolimkova, M., 2008. Gene polymorphisms of superoxide dismutases and catalase in diabetes mellitus. BMC Med. Genet. 9 (3).

Forsberg, L., de Faire, U., Morgenstern, R., 1999. Low yield of polymorphisms from EST Blast searching: analysis of genes related to oxidative stress and verification of the P197L polymorphism in GPx1. Hum. Mutat. 13, 294–300.

Förstermann, U., Gath, I., Schwarz, P., Closs, E.I., Kleinert, H., 1995. Isoforms of nitric oxide synthase. Properties, cellular distribution and expressional control. Biochem. Pharmacol. 50(9), 1321–1332 (Review).

Gallo, O., Masini, E., Morbidelli, L., Franchi, A., Fini-Storchi, I., Vergari, W.A., Ziche, M., 1998. Role of nitric oxide in angiogenesis and tumor progression in head and neck cancer. J. Natl. Cancer Inst. 90(8), 587–596.

Goldman, L., Ezzat, S., Mokhtar, N., Abdel-Hamid, A., Fowler, N., Gouda, I., Eissa, S.A., Abdel-Hamid, M., Loffredo, C.A., 2009. Viral and non-viral risk factors for non-Hodgkin's lymphoma in Egypt: heterogeneity by histological and immunological subtypes. Cancer Causes Control 20 (6), 981–987.

Hamanishi, T., Furuta, H., Kato, H., Doi, A., Tamai, M., Shimomura, H., Sakagashira, S., Nishi, M., Sasaki, H., Sanke, T., Nanjo, K., 2004. Functional variants in the glutathione peroxidase-1 (GPx-1) gene are associated with increased intima-media thickness of carotid arteries and risk of macrovascular diseases in Japanese type 2 diabetic patients. Diabetes 53 (9), 2455–2460.

He, C., Qureshi, A.A., Han, J., 2010. Polymorphisms in genes involved in oxidative stress and their interactions with lifestyle factors on skin cancer risk. J. Dermatol. Sci. 60 (1), 54–56.

Hu, D.E., Dyke, S.O., Moore, A.M., Thomsen, L.L., Brindle, K.M., 2004. Tumor cell-derived nitric oxide is involved in the immune-rejection of an immunogenic murine lymphoma. Cancer Res. 64 (1), 152–161.

Hung, R.J., Boffetta, P., Brennan, P., Malaveille, C., Gelatti, U., Placidi, D., Carta, A., Hautefeuille, A., Porru, S., 2004. Genetic polymorphisms of MPO, COMT, MnSOD, NQO1, interactions with environmental exposures and bladder cancer risk. Carcinogenesis 25 (6), 973–978.

Krajinovic, M., Sinnett, H., Richer, C., Labuda, D., Sinnett, D., 2002. Role of NQO1, MPO and CYP2E1 genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia. Int. J. Cancer 97(2), 230–236.

Lan, Q., Zheng, T., Shen, M., Zhang, Y., Wang, S.S., Zahm, S.H., Holford, T.R., Leaderer, B., Boyle, P., Chanock, S., 2007. Genetic polymorphisms in the oxidative stress pathway and susceptibility to non-Hodgkin lymphoma. Hum. Genet. 121 (2), 161–168.

Lightfoot, T.J., Skibola, C.F., Smith, A.G., Forrest, M.S., Adamson, P.J., Morgan, G.J., Bracci, P.M., Roman, E., Smith, M.T., Holly, E.A., 2006. Polymorphisms in the oxidative stress genes, superoxide dismutase, glutathione peroxidase and catalase and risk of non-Hodgkin's lymphoma. Haematologica 91(9), 1222–1227.

Liu, G., Zhou, W., Wang, L.I., Park, S., Miller, D.P., Xu, L.L., Wain, J.C., Lynch, T.J., Su, L., Christiani, D.C., 2004. MPO and SOD2 polymorphisms, gender, and the risk of non-small cell lung carcinoma. Cancer Lett. 214(1), 69–79.

London, S.J., Lehman, T.A., Taylor, J.A., 1997. Myeloperoxidase genetic polymorphism and lung cancer risk. Cancer Res. 57(22), 5001–5003.

Mannick, J.B., Asano, K., Izumi, K., Kieff, E., Stamler, J.S., 1994. Nitric oxide produced by human B lymphocytes inhibits apoptosis and Epstein–Barr virus reactivation. Cell 79(7), 1137–1146.

McElnea, E.M., Quill, B., Docherty, N.G., Irnaten, M., Siah, W.F., Clark, A.F., O'Brien, C.J., Wallace, D.M., 2011. Oxidative stress, mitochondrial dysfunction and calcium overload inhuman lamina cribrosa cells from glaucoma donors. Mol. Vis. 17, 1182–1191.

Mendes, R.V., Martins, A.R., de Nucci, G., Murad, F., Soares, F.A., 2001. Expression of nitric oxide synthase isoforms and nitrotyrosine immunoreactivity by B-cell non-Hodgkin's lymphomas and multiple myeloma. Histopathology 39 (2), 172–178.

Nemoto, M., Nishimura, R., Sasaki, T., Hiki, Y., Miyashita, Y., Nishioka, M., Fujimoto, K., Sakuma, T., Ohashi, T., Fukuda, K., Eto, Y., Tajima, N., 2007. Genetic association of glutathione peroxidase-1 with coronary artery calcification in type 2 diabetes: a case control study with multi-slice computed tomography. Cardiovasc. Diabetol. 7 (6), 23.

Olson, S.H., Carlson, M.D., Ostrer, H., Harlap, S., Stone, A., Winters, M., Ambrosone, C.B., 2004. Genetic variants in SOD2, MPO, and NQO1, and risk of ovarian cancer. Gynecol. Oncol. 93 (3), 615–620.

Park, H.H., Ha, E., Uhm, Y.K., Jin, S.Y., Kim, Y.J., Chung, J.H., Lee, M.H., 2006. Association study between catalase gene polymorphisms and the susceptibility to vitiligo in Korean population. Exp. Dermatol. 15 (5), 377–380.

Rajp, A., Adu, D., Savage, C.O., 2007. Meta-analysis of myeloperoxidase G-463/A polymorphism in anti-neutrophil cytoplasmic autoantibody-positive vasculitis. Clin. Exp. Immunol. 149 (2), 251–256.

Ratnasinghe, D., Tangrea, J.A., Andersen, M.R., Barrett, M.J., Virtamo, J., Taylor, P.R., Albanes, D., 2000. Glutathione peroxidase codon 198 polymorphism variant increases lung cancer risk. Cancer Res. 60(22), 6381–6383.

Ravn-Haren, G., Olsen, A., Tjønneland, A., Dragsted, L.O., Nexø, B.A., Wallin, H., Overvad, K., Raaschou-Nielsen, O., Vogel, U., 2006. Associations between GPX1

Pro198Leu polymorphism, erythrocyte GPX activity, alcohol consumption and breast cancer risk in a prospective cohort study. Carcinogenesis 27 (4), 820–825.

Shen, J., Wang, R.T., Wang, L.W., Xu, Y.C., Wang, X.R., 2004. A novel genetic polymorphism of inducible nitric oxide synthase is associated with an increased risk of gastric cancer. World J. Gastroenterol. 10(22), 3278–3283.

Skibola, C.F., Curry, J.D., Nieters, A., 2007. Genetic susceptibility to lymphoma. Haematologica 92 (07), 960–969.

Smedby, K.E., Lindgren, C.M., Hjalgrim, H., Humphreys, K., Schöllkopf, C., Chang, E.T., Roos, G., Ryder, L.P., Falk, K.I., Palmgren, J., Kere, J., Melbye, M., Glimelius, B., Adami, H.O., 2006. Variation in DNA repair genes ERCC2, XRCC1, and XRCC3 and risk of follicular lymphoma. Cancer Epidemiol. Biomark. Prev. 15 (2), 258–265.

Stuehr, D.J., 1999. Mammalian nitric oxide synthases. Biochim. Biophys. Acta 1411(2–3), 217–230.

Swerdlow, S.H., Campo, E., Harris, N.L., Jaffe, E.S., Pileri, S.A., Stein, H., Thiele, J., Vardiman, J.W. (Eds.), 2008. World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues. IARC Press, Lyon.

Tome, M.E., Johnson, D.B., Rimsza, L.M., Roberts, R.A., Grogan, T.M., Miller, T.P., Oberley, L.W., Briehl, M.M., 2005. A redox signature score identifies diffuse large B-cell lymphoma patients with a poor prognosis. Blood 106(10), 3594–3601.

van de Sande, W.W., Fahal, A., Verbrugh, H., van Belkum, A., 2007. Polymorphisms in genes involved in innate immunity predispose toward mycetoma susceptibility. J. Immunol. 179(5), 3065–3074.

Varma, S., Menon, M.C., Garg, A., Malhotra, P., Sharma, A., Chawla, Y.K., Dhiman, R.K., 2011. Hepatitis C virus infection among patients with non-Hodgkin's lymphoma in northern India. Hepatol. Int. 5 (2), 688–692.

Wang, L.I., Miller, D.P., Sai, Y., Liu, G., Su, L., Wain, J.C., Lynch, T.J., Christiani, D.C., 2001. Manganese superoxide dismutase alanine-to-valine polymorphism at codon 16 and lung cancer risk. J. Natl. Cancer Inst. 93(23), 1818–1821.

Wang, S.S., Davis, S., Cerhan, J.R., Hartge, P., Severson, R.K., Cozen, W., Lan, Q., Welch, R., Chanock, S.J., Rothman, N., 2006. Polymorphisms in oxidative stress genes and risk for non-Hodgkin lymphoma. Carcinogenesis 27 (9), 1828–1834.

Wang, S.S., Slager, S.L., Brennan, P., Holly, E.A., De Sanjose, S., Bernstein, L., Boffetta, P., Cerhan, J.R., Maynadie, M., Spinelli, J.J., Chiu, B.C., Cocco, P.L., Mensah, F., Zhang, Y., Nieters, A., Dal Maso, L., Bracci, P.M., Costantini, A.S., Vineis, P., Severson, R.K., Roman, E., Cozen, W., Weisenburger, D., Davis, S., Franceschi, S., La Vecchia, C., Foretova, L., Becker, N., Staines, A., Vornanen, M., Zheng, T., Hartge, P., 2007. Family history of hematopoietic malignancies and risk of non-Hodgkin lymphoma (NHL): a pooled analysis of 10 211 cases and 11 905 controls from the International Lymphoma Epidemiology Consortium (InterLymph). Blood 109(8), 3479–3488.