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Arsenic and Chromium levels in hair correlate with Actinic Keratosis/ Non Melanoma Skin Cancer: results of an Observational Controlled Study.

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ABSTRACT:

BACKGROUND: The role of heavy metals in carcinogenetic process has been widely established however, information on the most common environmental metals that serve as major risk factors for actinic keratosis (AK)/non-melanoma skin cancer (NMSC) are still lacking. We aim to evaluate levels of the most common environmental heavy metals in hair of patients with AK/NMSC as compared to healthy controls.

METHODS: 31 patients diagnosed with AK/NMSC and 34 healthy controls were enrolled. Patients were interviewed for heavy metals exposure and underwent hair analysis for detection of arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni) and lead (Pb). Continuous variables were analyzed using Wilcoxon non-parametric rank test and proportions were compared by Fisher exact test. Statistical significance was determined by $p<0.05$.

RESULTS: In our cohort we observed 48.4% patients had AKs, 16.1% basal cell carcinoma (BCC), 9.7% squamous cell carcinoma (SCC) and 25.9% of patients presented with a combination of these lesions. There were significantly elevated levels of As and Cr in AK-NMSC group as compared to controls.

CONCLUSIONS: we identified a strong positive correlation between As and Cr concentration and AK/NMSC adding new clues to the scenery of NMSC risk factors that should be taken under consideration in exposed populations.

KEY WORDS: heavy metals; actinic keratosis, non-melanoma skin cancer; arsenic; chromium

TEXT

Introduction

Non Melanoma Skin Cancer (NMSC) is th.e most frequent group of malignant tumors of the skin. They include basal cell carcinoma (BCC) and squamous cell carcinoma (SCC).[1] Recently, actinic keratoses (AK) have been considered as *in situ* SCC that can progress to invasive SCC, and is strongly associated with the onset of BCC and SCC.[2 3] UV light exposure is the most important environmental pathogenic factor in NMSC, with cumulative high doses predisposing to SCC and AK, and irregular exposure predisposing for BCC.[4 5] Moreover, other environmental exposures namely pollutants, ionizing radiations and heavy metals are also possible pathogenic factors for AK/NMSC.[6] Previous studies in animals and humans have shown that exposure to arsenic (As) and copper (Cu) may be associated with an increased risk for various skin cancers.[7-9]

In a hairless mice model, heavy metals including Ni and Cr were shown to synergistically enhance UV carcinogenesis, as compared to ingestion of Cr alone.[7] Carcinogenic activity of Cr likely did not involve oxidative mechanisms, as antioxidants Vitamin E or selenomethionine had no effect on its potentiating ability.[10] Recently, observational studies have also suggested a correlation between As exposure and AK/NMSC, with low concentrations of sodium arsenite shown to enhance tumorigenesis of solar UV irradiation in mice.[7 11] Previous studies associated exposure to As in dust and fumes with an increased risk for both BCCs and SCCs, however other heavy metals have no specific evidence of being linked to a major risk of developing AKs or NMSCs.[12] Cd also shows a correlation with higher incidence of malignancies, but a direct connection to AK is still not proven.[8] To better understand the role of heavy metals concentration in patients with AK-NMSC, we

conducted a case-control study in order to analyze As, Cd, Cr, Ni, and Pb levels in hair samples of patients affected by NMSCs. Human scalp hair has received attention recently as perfect sample for heavy metal analysis because it is not invasive, easier to collect and less prone to degradation compared to other biological specimens. Furthermore, elements have longer residence times and are more concentrated in scalp hair.[13] Human hair samples have been effectively utilized as biomarkers for the determination of trace element concentrations in environmental work.[14] The biological analysis of heavy metals pollution for human exposure has been often conducted on hair analysis, which has been understood to play an important role in the monitoring of long-term exposures.[15-17] Hair is now considered one of the most important biomarkers according to the Environmental Protection Agency (EPA). [18]

Materials and Methods

Study design

31 patients and 34 healthy controls were enrolled in this study. Patients diagnosed with AK or NMSC were consecutively enrolled from January 2017 to February 2018 at the Dermatology outpatients' clinic, Unit of Dermatology, Health Sciences Department, University "Magna Græcia" of Catanzaro. Each patient underwent hair analysis for detection of heavy metal contamination. Inclusion criteria were patients with Fitzpatrick photo-type 3. Exclusion criteria included inadequate hair length; lack of hair due to any condition. Patients' usages of hair products were reported. All subjects signed inform consent and were interviewed for occupational exposure, housing location (coastal or industrial area), smoking habits (any number of cigarettes per day), consumption of alcohol and/or seafood (more than twice a week) containing high amounts of arsenic or other metals, as well as dental implants and hair dyes.

Sample collection preparation and analysis

Analysis of heavy metals in hair included As, Cd, Cr, Ni and Pb as previously described.[16 19] Strands of hair (~ 1g) around 1-1.5cm were cut from the nape of the neck, as close as possible to the occipital scalp region. Ceramic scissors were used to prevent metal contamination. Immediately after, hair samples were sealed in their designated plastic bags with proper identifiers. Samples were prepared by washing with demineralized water and acetone ultrapure for three repeated cycles to eliminate external contamination such as sebum, sweat, and spray. The samples were then dried in oven at 40°C for 24h, followed by acid digestion. Aliquots of 0.5g of hair were placed in vessels and 6 ml of HNO_3 (65%) and 4 ml of H_2O_2 (30%) were added to each vessel.[20] All samples were digested in close vessels using Multiwave 3000 (Antoon Paar)[21] and analyzed by ICP-MS (inductively coupled plasma mass spectrometry) X Series II (Thermo Scientific) using external calibration. ICP-MS allows quantification of metals and several non-metals at concentrations as low as one part in 10. The lower detection limit was achieved by ionizing the sample with inductively coupled-plasma; the ions obtained were separated by a quadrupole mass spectrometer.

Statistical analysis

Continuous variables were analyzed using Wilcoxon non-parametric rank test and proportions were compared by Fisher Exact Test. Continuous variables are summarized as the median and interquartile ranges, while proportions are expressed as percentages. Statistical significance was determined by $p<0.05$.

Results

31 outpatients with AK-NMSC were included in the study, consisting of 16 women and 15 men (ages 68-77; mean 74) Table 1. Diagnosis was performed by clinical examination by two different dermatologists and confirmed by diagnostic punch biopsies. In total, 15 (48.5%) patients had solely AKs, 5 (16.1%) patients had only BCCs and 3 (9.6%) patients had only SCCs. Moreover, 5 (16.1%) patients had a combination of AKs and BCCs, 2 (6.5%) patients were diagnosed with AK and SCC and 1 (3.2%) patient had AK, BCC, and SCC. 34 healthy controls (HC) were included in the study, consisting of 19 women and 15 men (age range (55-76); mean 63) (Table 1). Age resulted to be different between NMSC and healthy control cohort (Table 1; $p<0.05$). To avoid bias due to the age difference we performed a sub-analysis with a smaller number of healthy controls showing no significant difference in our results (Supplementary Table E1). Smoking habit was significantly higher in the HC cohort (35%) as compared to the AK-NMSC cohort (9.7%) ($p=0.019$). No other significant differences were found in demographics data between AK-NMSC patients and HC subjects (Table 2).

We also considered for common exposures to environmental toxicants. We found no significant differences between HC and AK-NMSC subjects regarding consumption of alcohol or sea food, usage of amalgam contained in dental implants, hair dye, residential location near industries or coastal regions, and occupational exposures (Table 2).

We analyzed metals in human hair including As, Cd, Cr, Ni, and Pb. The mean concentrations (+/- SD) in AK-NMSC patients and in HC subjects are listed in Table 3. Among the metals studied, only As and Cr levels were significantly higher in AK/NMSC patients as compared to HC subjects (Table 3). There were no significant differences between AK-NMSC subjects and HC subjects in level of Cd, Pb, and Ni (Table 3).

Discussion

The association between chemical agents and skin cancer has been proposed for centuries. In 1775, Percival Pott observed a high incidence of scrotum squamous cells carcinoma among the London chimney sweepers.[22] Other observation at the beginning of 19th Century associated SCC with exposure to toxic compounds, ~~such as As based arsenide~~ in Paris sewers.[23] Various malignancies and skin conditions have also been reported in chronic arsenicism.[24 25] An increased risk of cancer was found in patients treated with As containing compounds (ie. Fowler Solution, 1% potassium arsenite) for psoriasis and other diseases.[26,27] Tseng and his collaborators found a strong correlation between prevalence of skin cancer in southwestern Taiwan and the residents exposure to As containing artesian wells.[28] Risk of developing skin cancers have also been stratified based on level of As contamination in drinking water[29-31]. In mild level of As contamination (<300 µg/L) incidence of skin cancer is 2.6 per 1000 people achieving 21.4 per 1000 people in areas with high level of arsenic contamination (>600 µg/L).[29] Although the carcinogenic role of some heavy metal has been investigated, correlations with skin carcinogenesis of a panel of heavy metals in a well characterized population is missing.

Our study investigated As, Cd, Cr, Ni, and Pb levels in hair of patients with AK/NMSC as compared to healthy subjects of similar demographic background. Statistical analysis showed that only As and Cr levels were significantly higher in AK-NMSC patients as compared to controls. The presence of high doses of As in patients with cutaneous carcinoma corresponds to previous literatures.[6 30 32] On the contrary, Cr may represent a new research perspective, as, at the best of our knowledge, there are no studies that showed a correlation between AK-NMSCs and exposure to Cr. Cr is designated as a group I carcinogen to humans by IARC, since its exposure is related to lung carcinomas and paranasal sinuses carcinomas.[33-34] Cr is used in industries for the production of automobiles, glass, ceramics, linoleum, and can sometimes be present in drinking water.[35-36] Ingestion of

liquids containing Cr causes severe gastroenteritis with nausea, electrolyte disturbances, shock and acidosis along with respiratory diseases such as ulcerative rhinitis, chronic bronchitis.[37] Some studies consider hexavalent Cr as a possible risk factor for melanoma, where patients with concurrent orthopedic prostheses and melanoma were found to have two to ten times higher level of Cr and cobalt as compared to the healthy population.[37-38]

Although Cr is currently not considered as a probable risk factor for the onset of cutaneous carcinomas, it does have a particular tropism for skin, hair, and nails.[39] In this study we correlated the increased level of Cr with the possibility to develop AK/NMSC. According to normal toxicokinetics, inorganic arsenic is detoxified through a methylation process by arsenic-methyltransferase. This enzyme catalyzes the conversion of iAs into methyl-arsenite and dimethyl-arsenite.[27] Numerous studies have confirmed that polymorphisms of GST, an enzyme that catalyzes the link between glutathione and its substrate, such as GST-M1, T1 and P1, are associated with the onset of cutaneous carcinomas.[40] Furthermore, recent studies evaluating the association of GST genetic polymorphisms and As exposure in the onset of cutaneous carcinomas have identified increased susceptibility in certain isoforms of GST to the carcinogenic effects of heavy metals.[41] It can be hypothesized that high levels of As and chromium Cr in patients with AK/NMSC compared to controls may be attributed to altered metabolism of these metals. The particular tendency of arsenic to accumulate in the skin and the presence of polymorphisms of GST can possibly explain the high levels of arsenic among the diseased subjects. Other hypothesis linking detoxification pathways and cancer developments see involve Cr metabolism.

Hexavalent Cr is the known carcinogenic isoform of the metal and is reduced by ascorbate and reduced GSH to tetravalent Cr, subsequently to non-toxic trivalent Cr that gets eliminated through urine (56%) and feces (5%).[42] Since hexavalent Cr and As are detoxified by the action of GSH, the functionally altered forms of GSH-binding proteins could partially

explain the high levels detected in patients with cutaneous carcinomas. However, the presence of the aforementioned polymorphisms does not fully explain the elevations of these metals in patients with AK/NMSC and future studies should explore alternative mechanisms for detoxification of other heavy metals. Although the high level of As and Cr in patients could be due to an altered metabolism leading to higher retention, the main issue might be represented by a high intake of such metals from uninvestigated sources. Population studies on broader environmental pollutant and living habits are needed to shade a light in the mechanism leading to a selective retention of specific metals in affected individuals.

We identified a significant difference in level of Cr and As in patients with AK/NMSC as compared to healthy controls, with significant strong positive correlation between these heavy metal exposure and AK-NMSCs. Thus, environmental pollution factors different from those investigated in our study may be additional contributing factors in predisposing our patients to higher risk of cancer formation. It can be postulated that patients with AK-NMSC may have either altered mechanisms of detoxification that impairs their ability to recover from toxic agent insults, or an augmented exposure to environmental pollutants.

Conclusions

Our study expands the current knowledge on the carcinogenic roles of heavy metals, especially considering the heavy use of Cr in industries. Our results, based on a comparable case-control population, shows that regardless the possible etiologic hypothesis leading to heavy metals accumulation, not only As but also Cr are associated with cutaneous carcinogenesis. Our data propose that not only As but also high intake or overexposure to Cr should be considered as an independent risk factor for AK/NMSC. Future studies should

investigate larger sample, different age and ethnicity cohorts, and attempt to elucidate the pathogenic role of exposure to environmental agents in skin cancer development.

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NOTES

Conflicts of interest.— The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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TABLES:

Table1: Demographic characteristics . AK-NMSC= actinc keratosis/non melanoma skin cancer ; AK= actinic Keratosis; BCC= basal cell carcinoma; SCC= squamous cell carcinoma; HC= healthy controls. +p<0.1; *p<0.05; **p<0.01; ***p<0.001.

BIOMETRIC AND CLINICAL DATA		
	AK/NMSC	**HC**
Subjects number	31	34
Age (years)	74 (68-77)	63 (55-76) *
M/F	16/15 (51.6%)/(48.4%)	19/15 (55.9%)/(44.1%)
AK	15 (48.4%)	-
BCC	5 (16.1.%)	-
SCC	3 (9.6%)	-
AK+ SCC	2(6.5%)	-
AK+ BCC	5 (16.1.%)	-
AK+ SCC+ BCC	1 (3.2%)	-

Table 2: anamnestic data. AK-NMSC= actinc keratosis/non melanoma skin cancer; HC= healthy controls. +p<0.1; *p<0.05; **p<0.01; ***p<0.001.

ANAMNESTIC DATA			
	AK-NMSC	**HC**	**p value**
Subjects #	31	34	
Residence on coast	9 (29%)	8 (23.5%)	0.78
Residence near industrial area	3 (9.7%)	2 (5.9%)	0.66
Professional exposure	4 (12.9%)	6 (17.6%)	0.74
Smoking habit	3 (9.7%)	12 (35.3%)	0.019 *
Alcohol consumption	10 (32.3%)	6 (17.6%)	0.25
Seafood consumption	2 (6.5%)	6 (17.6%)	0.26
Dental implants	12 (38.7%)	15 (44.1%)	0.80
Hair dye	10 (32.3%)	16 (47.1%)	0.31

Table 3: Heavy metal concentration in human hair (mcg/g). arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni) and lead (Pb). IQR=Interquartile range; +p<0.1; *p<0.05; **p<0.01; ***p<0.001.

Heavy metal concentration in human hair (mcg/g)

	AK/NMSC	HC	p value	Fold-change
As (median, IQR)	0.119 (0.073-0.17)	0.01 (0.009-0.028)	< 0.001 ***	11.9
Cr (median, IQR)	0.093 (0.08-0.22)	0.07 (0.04-0.11)	0.009 **	1.33
Cd (median, IQR)	0.008 (0.006-0.02)	0.01 (0.01-0.02)	0.43	0.8
Ni (median, IQR)	0.154 (0.13-0.26)	0.11 (0.07-0.20)	0.025 *	1.4
Pb (median, IQR)	0.53 (0.40-0.86)	0.44 (0.22-0.89)	0.13	1.2

Supplementary Table E1: Sub-analysis (mean Age controls 71.4 vs mean Age NMSC 71.5, p=0.95)

Heavy metal concentration in human hair (mcg/g)					
	AK/NMSC	HC	p value		Fold-change
As (median, IQR)	0.119 (0.073-0.17)	0.01 (0.006-0.02)	< 0.001	***	11.89
Cr (median, IQR)	0.093 (0.08-0.22)	0.07 (0.3-0.1)	0.003	**	1.55
Cd (median, IQR)	0.008 (0.006-0.02)	0.01 (0.005-0.02)	0.95		0.8
Ni (median, IQR)	0.154 (0.13-0.26)	0.11 (0.07-0.15)	0.007	**	1.54
Pb (median, IQR)	0.53 (0.40-0.86)	0.44 (0.24-0.84)	0.36		1..06