Elevated levels of Soluble CTLA-4, PD-1, PD-L1, LAG-3, and TIM-3, and Systemic Inflammatory Stress as potential contributors to immune suppression and generalized Tumorigenesis in a cohort of South African Xeroderma Pigmentosum patients

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Background

- Xeroderma Pigmentosum (XP), an autosomal recessive disorder characterized by ultraviolet radiation-induced abnormalities of DNA excision and repair pathways is
- associated with the early development of cutaneous cancers.
- Intracellular oxidative stress has also been proposed as a contributor to the occurrence of skin cancers.
- Little is known about the possible augmentative contributions of chronic inflammation, immune suppression, and oxidative stress to the pathogenesis of malignancies associated with other subtypes of XP.

Methods

- The study population consisted of a total of 23 South African XP patients attending the Dermatology Screening Clinic at Steve Biko Academic Hospital, Pretoria, South Africa (Table 1). All results were compared to healthy controls (Table 2).
- Whole venous blood samples were collected in ethylenediaminetetraacetic acid (EDTA) vacutainers on different dates in two batches of varying sizes during September 2019 and November 2020 and processed promptly to separate the plasma component by centrifugation and stored at minus 70°C.
- Biomarkers measured in XP patients and healthy controls included the cytokines, interleukins (ILs)-2, -4, -6, -10, interferon-g (IFN- g), and tumor necrosis factor-a (TNF-a), C-reactive protein (CRP), and cotinine.
- These biomarkers were measured in plasma using immunofluorimetric, nephelometric, and ELISA procedures.
- Informed consent was obtained from all the adult patients and from the parents and guardians of the affected children, as well as from the healthy control participants mentioned below, all of whom fully understood the purpose of the study, which was undertaken in full compliance with the 1964 Declaration of Helsinki.
- Ethics approval was granted by The Research Ethics Committee, Faculty of Health Sciences, University of Pretoria (Ethics Committee Approval Numbers 326/2016, 251/2019 and 510/ 2020).

Results

- Immune suppression was detected according to the levels of five soluble inhibitory immune checkpoint molecules (ICM) (CTLA-4, PD-1, PD-L1, LAG-3, and TIM-3), as well as those of vitamin D, while oxidative stress was determined according to the circulating levels of the DNA adduct, 8-hydroxy-2-deoxyguanosine (8-OH-dG).

Table 1. Phenotypic and genotypic characteristics of XP patients.

		Age		Clinical manifestations		
		onset	current	skin	mouth	eye involvement
XPC families						
03	F	18 m	19 y	BCC SCC	tongue/lip	keratopathy/tumor
04	F	6 m	18 y	BCC SCC	tongue	enucleation/tumor
06	F	9 m	10 y	BCC SCC	tongue	corneal scarring
08	M	2 y	6 y	BCC SCC	tongue	keratopathy
10	M	2 y	8 y	BCC SCC	tongue	corneal scarring
11	F	3 y	16 y	BCC SCC	tongue	corneal scarring
12	F	2 y	8 y	BCC SCC	tongue/lip	keratopathy
14	M	-	3 y	none	none	photophobia
15	M	1 y	10 y	BCC SCC	tongue	corneal scarring
16&17	M	2 y	9 y	BCC SCC	tongue	keratoconjunctivitis
18	F	1 y	6 y	BCC SCC	none	fibrosis
19	F	9 m	6 y	BCC SCC	lip	eyelid tumor
XPD families						
05	M	-	4 y	freckling/sun sensitivity	none	keratopathy/enucleation
09	F	-	10 y	freckling/sun sensitivity	none	photophobia/MR
XPE family						
01	F	-	51 y	freckling/sun sensitivity	none	none
02	F	-	48 y	freckling/sun sensitivity	none	none
Other families						
07	F	Birth	4 y	freckling/skin xerosis	none	none
13	F	-	6 y	freckling	none	none
20	M	2 y	5 y	SCC	tongue/lip	photophobia
21	F	8 m	2 y	SCC	none	keratopathy/tumor/corneal scarring
22	M	3 y	9 y	SCC	tongue/lip	tumor/photophobia
23	M	3 y	7 y	freckling/actinic keratosis	Actinic cheilitis	photophobia

F, female; M, male; m, month; y, year; BCC, basal cell carcinoma; SCC, squamous cell carcinoma; MR, mental retardation.

Table 2. Control group characteristics

Analysis	Patients	Male	Female	Ethnicity	Age (mean)
8-hydroxy-2- deoxyguanosine (8-OH-dG) Non-smoking	6	0	6	African	30.3 ± 5.5 years
8-hydroxy-2- deoxyguanosine (8-OH-dG) Smoking	6	5	1	African	29.2 ± 4.3 years
Cytokines (all non-smoking)	15	6	9	African	32.5 ± 4.8 years
Immune Checkpoints	5	2	3	African	25.0 ± 6.3 years
Vitamin D	2	0	2	Caucasian	53 ± 7.1 years

Table 3. Plasma concentrations of 8-hydroxy-2-deoxyguanosine (8-OH-dG) in patients with XP and sub-groups of healthy non-smoking and smoking control subjects.

Group	Plasma concentrations of 8-OH-dG (pg/mL)
XP patients (n=19)	4701.56 (4085.27-5697.42)*
Non-smoking controls (n=6)	7078.80 (5831.85-9233.74)
Smoking controls (n=6)	6996.42 (6352.05-8418.11)
Combined group of control subjects (n=12)	6996.42 (5859.97-8838.92)+

**Results expressed as the median values in pg/mL plasma with 25% and 75% IQRs.*
+P<0.001 for comparison of the XP patient group with the combined group of control subjects.

Table 4. Comparison of the plasma levels of TNF-a, IL-6 and IL-10 in the subgroups of XP patients with normal and elevated levels of CRP.

Cytokines (pg/mL)	XP Patients	
	Elevated CRP (n=8)	Normal CRP (n=11)
TNF -α	7.66 (6.75-11.43)*	6.27 (5.56-7.11)
IL-6	2.77 (2.17-4.43)	2.02 (1.92-2.27)
IL-10	1.59 (0.62-2.03)	0.62 (0.62-0.62)

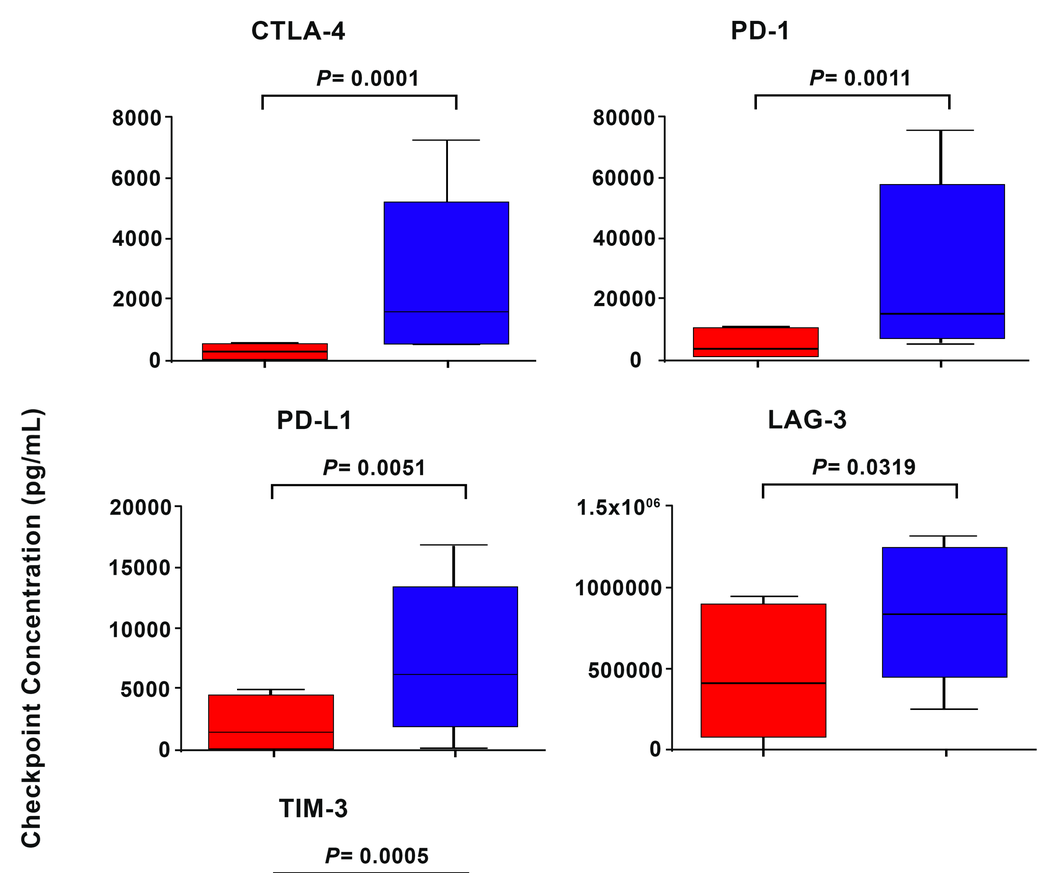
**Results expressed as the median values in pg/mL plasma with 25% and 75% IQRs.*

Table 5. Comparison of the concentrations of the test soluble inhibitory immune checkpoints in plasma samples from control participants and Xeroderma Pigmentosum patients.

Checkpoints	Control Participants (n=5)	Xeroderma Pigmentosum Patients (n=15)	*P* ≤
CTLA-4	418.42 (310.17–451.39)*	1550.09 (612.46–2891.79)	0.0001
PD-1	7664.25 (4364.5–9932.47)	16480.11 (11163.3–34197.63)	0.001
PD-L1	1325.81 (271.92–3665.64)	6143.29 (3954.88–9192.49)	0.005
LAG-3	423768.0 (181603.8–843313.7)	839880.1 (671236.5–1150400.0)	0.032
TIM-3	2687.71 (2594.45–3496.56)	6648.43 (5670.46–9712.61)	0.0005

**Results expressed as the median values in pg/mL plasma with 25% and 75% IQRs.*

Figure 1. Box and whisker plots showing a comparison of the plasma concentrations as pg/mL of the five soluble inhibitory immune checkpoints, CTLA-4, PD-1, PD-L1, LAG-3 and TIM-3 in control subjects relative to those of the cohort of XP patients.



Conclusions

- The findings of increased levels of pro-inflammatory cytokines and, in particular, those of the soluble ICM, in the setting of decreased vitamin D and moderately elevated levels of CRP in XP patients suggest a possible secondary role of ongoing, inflammatory stress and immune suppression in the pathogenesis of XP-associated malignancies.

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