

Status and Interrelationship of Zinc, Copper, Iron, Calcium and Selenium in Prostate Cancer

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Abstract Deficiency or excess of certain trace elements has been considered as risk factor for prostate cancer. This study was aimed to detect differential changes and mutual correlations of selected trace elements in prostate cancer tissue versus benign prostatic hyperplasia tissue. Zinc, copper, iron, calcium and selenium were analysed in histologically proven 15 prostate cancer tissues and 15 benign prostatic hyperplasia tissues using atomic absorption spectrophotometer. Unpaired two tailed *t* test/Mann–Whitney U test and Pearson correlation coefficient were used to compare the level of trace elements, elemental ratios and their interrelations. As compared to benign prostatic tissue, malignant prostatic tissue had significantly lower selenium ($p = 0.038$) and zinc ($p = 0.043$) concentrations, a lower zinc/iron ratio ($p = 0.04$) and positive correlation of selenium with zinc ($r = 0.71$, $p = 0.02$) and iron ($r = 0.76$, $p = 0.009$). Considerably divergent interrelationship of elements and elemental ratios in prostate cancer versus benign prostatic hyperplasia was noted. Understanding of differential elemental changes and their interdependence may be useful in defining the complex metabolic alterations in prostate carcinogenesis with potential for development of element based newer diagnostic, preventive and therapeutic strategies. Further studies may be needed to elucidate this complex relationship between trace elements and prostate carcinogenesis.

Keywords Prostate cancer · Trace elements · Benign prostatic hyperplasia · Prostate carcinogenesis, metal carcinogenesis · Prostate cancer prevention

Introduction

It is well known that some of the heavy metals are essential while some have toxic and carcinogenic effects in humans [1]. Studies on concentration of these trace metals in human play an important role in better understanding of biochemical and metabolic processes occurring in cells. Instability (overabundance or deficiency) in the composition of these trace metals may play a critical role in cancer biology; however, their precise role in initiation, promotion and inhibition of carcinogenic process is still undefined.

For understanding the relationship between prostate cancer and metals, it may be important to identify differences in homeostasis of trace elements between two common diseases of aging prostate i.e. benign prostatic hyperplasia (BPH) and prostate cancer (PCa). As both of these conditions have different histopathology and clinical behaviour, different metabolic alterations should account for these pathological processes. Alterations in levels of certain trace elements especially selenium and zinc have been identified in prostate cancer [2–5]. However, administration of Se to subjects in clinical trials [6] and Zn in case control studies [7] failed to prevent the prostate cancer. Thus, in addition to absolute tissue levels of various metals, their ratios and mutual correlations may help in understanding the complex metabolic dynamics of trace elements in prostate cancer. Only isolated studies have examined this complex interrelationship [8, 9] or ratios [10] of the trace elements in prostate cancer tissue. The aim of present study was to assess differences in levels, ratios

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and inter-relationship of five elements i.e. Copper (Cu), Calcium (Ca), Iron (Fe), Zinc (Zn) & Selenium (Se) between benign versus malignant prostate tissue. The understanding of this disturbed homeostasis of trace elements in cancerous tissue may have significant implications for development of element related preventive or therapeutic interventions for prostate cancer.

Materials and Methods

This study was approved by the institutional review board and ethics committee of our institution. Informed consent was obtained from all individual participants included in the study.

Patients and Protocol

The study was conducted on prostatic tissues obtained from 30 patients who attended our tertiary care teaching institution for surgical management of BPH (group 1 = 15 patients) and prostate cancer (group 2 = 15 patients). Exclusion criteria included diabetes, any other malignancy, any hormone therapy or steroid therapy. In BPH group, transurethral resection (TUR) chips were used where as in prostate cancer group either channel TUR chips or tissue obtained from radical prostatectomy specimens after sectioning the prostate were used. Parts of the selected tissues were separately sent to confirm the representative histopathology. Concentration of Zn, Cu, Fe and Ca was analysed in all 30 patients (15 BPH and 15 PCa samples) and that of Se was analysed in 20 patients (10 BPH and 10 PCa samples).

Tissue Preparation and Trace Elements Analysis

Fresh prostate tissues were washed in deionised water and at least 1 gram of the tissue (range 1.050–1.428 g) was carefully weighed and stored at -20°C . These samples were digested in a mixture of nitric: perchloric acid (6:1) in Kjeldahl flasks on slow heating. The heating was continued till white fumes evolved and the volume was reduced to 0.5–1.0 ml. The contents were made up to 10 ml with 0.1 N nitric acid, filtered and used for the analysis of zinc, copper, iron and calcium on a flame Atomic Absorption Spectrometer (AAS) (Perkin Elmer, AAnalyst 300). Selenium was analysed on an AAS equipped with a Graphite tube atomiser (Analytik Jena, Zeenit 700). The method was validated using the matrix spiking, recovery experiments, repeatability, reproducibility, limit of detection (LOD) and limit of quantitation (LOQ). Reagent blanks were also prepared and processed in the same manner for analysis. Recovery experiments indicated that the percentage

recovery ranges from 80–120 % and the instrument calibration was done using the NIST traceable certified reference material (ICP multi-element standard solution IV from Merck, Darmstadt, Germany). Concentration of Zn, Cu, Fe and Ca are reported in $\mu\text{g/g}$ while Se is reported in ng/g of wet tissue. Trace element analysis was done at Analytic Chemistry Laboratory, CSIR - Indian Institute of Toxicology Research, Lucknow, India. The following sequence was followed for the analysis on the guidelines of MET-CHEM-AA-01 and MET-CHEM-AA-02 as described by Neugebauer et al. [11]

Analysis sequence performed:

Solution type	Acceptance criteria and comments
Calibration blank	To check the background contamination. There should not be any contamination
Sensitivity check standard	For determination of LOD and LOQ of the instrument
Standards (CRMs)	To check the linearity range of the instrument
Calibration blank	No contamination should be there in blank
Calibration verification standard	It should read between 80 and 120 %
Reagent blank	Should be lower than 10 times the acceptable limit of error for that particular analyte
Standard reference material	By matrix spiking. Recovery between 80 and 120 %
Establishment of LOD/LOQ for the method used	By replicate analysis of spiked samples at ppb level and after getting the LOQ value, practically demonstrated to get confidence in the method used
Samples	Absorbance should be <highest standard % RSD of two readings should be $\pm 20\%$ Recovery of spiked sample should be 80–120 % Values less than LOQ evaluated
SRM and calibration blank	Used for checking the instrumental performance in between the sample analysis

Statistical Analysis

The results are presented as mean \pm SD. The unpaired t-test/Mann–Whitney U test was used to compare the various elements, ratios of elements, age and PSA between BPH and Cap groups. The Pearson correlation coefficient was calculated among the parameters to find the trend of

correlation i.e. any correlation of trace elements with either each other in benign and cancerous tissues or other patient parameters i.e. age, ultrasonographic prostate size (in case of BPH) and PSA (in case of prostate cancer). The *p* value <0.05 was considered to be significant. All the statistical analysis was carried out using SPSS 16.0 version (Chicago, In., USA).

Results

Mean age was similar in both PCa and BPH groups (Table 1). Selenium and Zinc levels were significantly (*p* = 0.038 and *p* = 0.043 respectively) lower in PCa tissue as compared to BPH tissue. Levels of Cu, Ca and Fe were not statistically different (*p* = 0.23, 0.47 and 0.93) between PCa and BPH. On comparing the ratios of trace elements, Zn/Fe ratio was significantly (*p* = 0.04) lower whereas Fe/Se and Ca/Se ratios were slightly higher (*p* values 0.068 and 0.08 respectively) in PCa as compared to BPH.

Table 2 and Fig. 1 show the results of correlation statistics. On examining the independent correlations of elements, the Zn level was positively correlated with Ca, Fe, Cu and Se (*p* values = 0.005, 0.006, 0.019 & 0.02 respectively) in PCa, whereas in BPH it was positively correlated with Fe (*p* value = 0.035) only. In malignant tissues, Se level was also positively correlated (*p* value = 0.009) with Fe levels. On examining the independent correlations between various trace elements and their ratios, Zn/Fe ratio was found to be positively correlated (*p* = 0.028) with Ca/Cu ratio in PCa.

On comparing the correlation coefficients (Table 3) of elements between PCa and BPH, the correlation of Se with Fe was significantly different (*r* = -0.13 vs. 0.76, *Z*-score = 2.15, *p* = 0.03) in these two conditions. Among elemental ratios, Ca/Zn had significantly different correlations with Zn/Cu and Zn/Fe in PCa (no correlation) as compared to BPH (negative correlation). Further, Ca/Zn to Ca/Cu, Ca/Fe to Ca/Cu and Fe/Se to Cu/Se correlations were significantly different between PCa (strongly positive) and BPH (mild positive or no correlation).

In the PCa group, comparison of parameters between high grade (Gleason > 7, *n* = 5) versus non-high grade (Gleason ≤ 7, *n* = 10) tumors, showed no differences in age, tissue element levels and ratios of elements (*p* values >0.05). PSA was significantly (*p* < 0.0001) higher in high grade tumors as compared to low grade tumors. There was no correlation of element levels or their ratios with age, PSA (in PCa group) or size of prostate gland (in BPH group).

Discussion

Obtained concentration ranges for various elements in our study were similar to that reported in literature for wet benign and malignant prostatic tissues [10, 12, 13]. Of the five measured elements, a significant difference was noticed in the concentration of Se and Zn only between PCa and BPH groups. While higher levels of Fe and Cu have been reported in diet [14], studies noted an insignificant difference in the levels of these elements in prostatic tissue [10, 12], blood [15] and hair [16] of PCa patients as

Table 1 Comparison of age, levels of various elements, elemental ratios and PSA between benign prostatic hyperplasia (BPH) and prostate cancer (PCa)

Parameter/element/elemental ratio	BPH	PCa	<i>p</i> value
Age(years)	67.9 ± 8.09	63.9 ± 12.1	0.2
Ca (µg/g wet tissue)	161.8 ± 78.5	140.7 ± 81.6	0.47
Se(ng/g wet tissue)	245.7 ± 195.6	91.5 ± 95.3	0.038
Cu(µg/g wet tissue)	5.02 ± 1.87	4.05 ± 2.41	0.23
Zn(µg/g wet tissue)	77.9 ± 43.4	49.2 ± 29.3	0.043
Fe(µg/g wet tissue)	55.04 ± 31.8	53.9 ± 38.4	0.93
Zn/Se	535 ± 347	797.5 ± 715.5	0.31
Zn/Cu	16.2 ± 8.05	13.2 ± 4.77	0.21
Zn/Fe	1.58 ± 0.7	1.07 ± 0.4	0.04
Ca/Zn	2.73 ± 1.89	3.29 ± 1.91	0.42
Cu/Se	28.01 ± 18.9	79.6 ± 98.8	0.12
Ca/Cu	35.4 ± 18.9	45.4 ± 36.9	0.36
Fe/Cu	11.16 ± 5.62	14.07 ± 7.38	0.23
Ca/Se	1179 ± 939.8	2404.5 ± 1868.6	0.08
Ca/Fe	3.54 ± 2.13	3.86 ± 4.17	0.79
Fe/Se	328.3 ± 215.6	673 ± 518	0.068
PSA (ng/ml)	2.3 ± 0.94	40.7 ± 44.7	0.001

Table 2 Significant correlations between tissue level of element and their ratios—in benign prostatic hyperplasia (BPH) versus prostate cancer (PCa)

Element/elemental ratio	Significant correlations ($p < 0.05$)					
	In BPH			In PCa		
	Correlation with	r value	p value	Correlation with	r value	p value
Se	Cu/Se	−0.7	0.024	Zn	0.71	0.02
	Zn/Se	−0.7	0.023	Fe	0.76	0.009
	Ca/Se	−0.69	0.026	Ca/Se	−0.67	0.03
	Fe/Se	−0.66	0.037			
Zn	Fe	0.54	0.035	Fe	0.67	0.006
	Zn/Cu	0.77	0.001	Ca	0.67	0.005
	Ca/Zn	−0.67	0.006	Cu	0.59	0.019
Zn/Fe	Zn/Cu	0.55	0.033	Ca/Cu	0.56	0.028
	Ca/Zn	−0.57	0.026	Ca/Fe	0.73	0.002
				Fe/Cu	−0.63	0.011
				Fe	−0.55	0.033
Fe/Se	Fe/Cu	0.66	0.035	Cu/Se	0.93	0.000
	Zn/Se	0.71	0.020	Zn/Se	0.91	0.000
	Ca/Se	0.66	0.036			
	Fe	0.64	0.044			
Ca/Se	Ca/Cu	0.83	0.003	Ca/Fe	0.65	0.039
	Ca	0.74	0.013	Ca/Zn	0.67	0.031
				Zn/Se	0.65	0.040
Fe	Fe/Cu	0.77	0.001	Fe/Cu	0.78	0.001
	Ca/Fe	−0.52	0.044			
Zn/Se	Cu/Se	0.90	0.0003	Cu/Se	0.94	0.00003
Zn/Cu	Ca/Zn	−0.63	0.011	Ca/Cu	0.58	0.022
Ca/Cu	Ca/Fe	0.63	0.012	Ca/Fe	0.93	0.00001
	Ca	0.75	0.001	Ca/Zn	0.88	0.00002
				Cu	−0.518	0.048
Ca/Zn	–	–	–	Ca/Fe	0.85	0.00004

compared to BPH patients. In malignant prostatic tissue, some [10] reported a significantly lower Ca level, whereas others [12] reported a significantly higher Ca concentration as compared to BPH. Hence, our findings revealing insignificant differences in Fe, Cu and Ca concentration between PCa and BPH tissue conform to these reports.

Reports regarding the prostatic tissue selenium changes in PCa versus BPH have been somewhat conflicting. Guntupalli et al. [17] showed significantly lower Se levels in PCa and BPH both (vs. normal prostatic tissue) with more significant decrease in case of cancer. In contrast, Zachara et al. [18] found significantly ($p < 0.01$) higher Se level in PCa as compared to both healthy controls and benign prostate hyperplasia. Zaichick and Zaichick [8] found Se to be significantly higher in BPH tissue as compared to normal and malignant tissues. In our study, Selenium level was found to be significantly lower ($p = 0.01$) in PCa versus BPH. Although, observational epidemiological studies suggest an inverse association between selenium and risk of prostate cancer [19, 20],

randomized controlled trials of selenium supplementation have reported conflicting results [21, 22]. In the Selenium and Vitamin E Cancer Prevention Trial (SELECT) clinical trial, selenium supplementation had no effect on prostate cancer risk [23]. Rather it might increase the risk of high-grade PCa among men with high selenium status [24]. Among men with non-metastatic prostate cancer, there is suggestive evidence that genetic variation in selenoproteins and related antioxidant enzymes may be associated with risk of high-grade disease at diagnosis and prostate cancer recurrence [25].

We also noted a lower ($p = 0.043$) Zn level in malignant tissue similar to many studies showing a significantly lower tissue zinc levels in prostate cancer as compared to normal prostatic tissue [9, 10, 13, 17] or BPH tissue [9, 10, 13]. By contrast, isolated studies have reported a significantly higher tissue level of zinc in PCa as compared to BPH [12] and normal prostatic tissue [27] or an insignificantly lower level of zinc level in PCa as compared to normal prostatic tissue [28]. There are conflicting reports

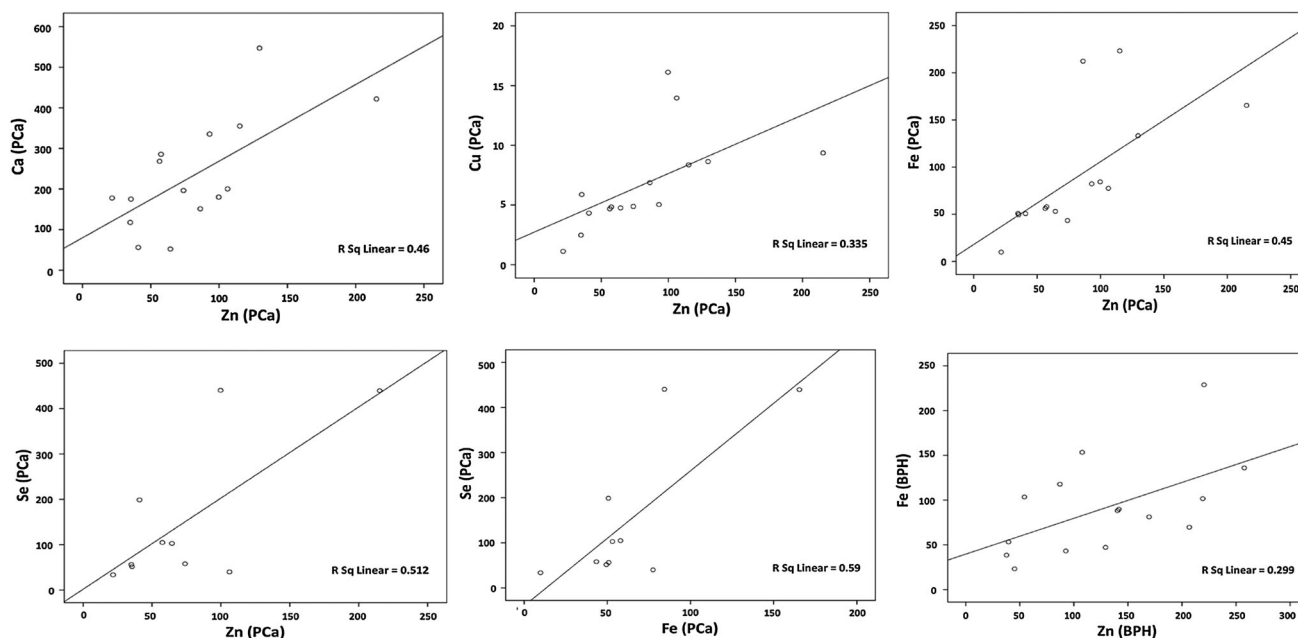


Fig. 1 Scatter plot diagrams showing significant ($p < 0.05$) correlations between trace elements in prostate cancer (PCa) and benign prostatic hyperplasia (BPH) (concentration of Zn, Ca, Cu and Fe is shown in $\mu\text{g/g}$ wet tissue and that of Se is shown in ng/g wet tissue)

Table 3 Significant differences on comparisons of correlation coefficients of elements and their ratios between benign prostatic hyperplasia (BPH) and prostate cancer (PCa)

Correlated elements or elemental ratios	Correlation coefficients in		Z-score	p value
	BPH	PCa		
Se & Fe	-0.13	0.76	2.15	0.03
Ca/Zn & Zn/Cu	-0.63	0.22	2.39	0.017
Ca/Zn & Zn/Fe	-0.57	0.37	2.55	0.01
Ca/Zn & Ca/Cu	0.44	0.88	2.25	0.024
Ca/Fe & Ca/Cu	0.63	0.93	2.24	0.025
Fe/Se & Cu/Se	0.56	0.93	2	0.045

about tissue zinc levels in BPH as well; some found a decrease [17] while others an increase [13] or no change [18, 26] as compared to normal prostatic tissue. The potential reasons for a variable difference in tissue Zn level between PCa and BPH in studies may be—unwanted possible representative tissue sampling errors or ethnic related. Despite the evidence [29] for decrease in serum and tissue Zn as well as significance of zinc in PCa in experimental studies, the epidemiological data have shown mixed results between zinc status and PCa, varying from direct [30–32] to inverse [33–35] correlation and no significant correlation [7, 36]. Higher levels of zinc

supplementation have been shown to increase prostatic tissue zinc levels and induce intraepithelial carcinoma prostate in murine model [37].

Inconsistency of Zn and inefficacy of Se in significantly altering the natural history of prostate cancer suggest that permissive, balancing and differential roles of various other elements may be equally important in the complex process of carcinogenesis. In order to probe this complexity of interdependence of various elements, we compared the ratios and interrelationship among various elements and elemental ratios in PCa versus BPH.

To the best of our knowledge, the only study available on the changes in ratios of metal ions in BPH or PCa is by Sapota et al. [10] for Zn, se, Cu, Fe and Ca. Of these, they reported 2.4 times lower Zn/Cu ratio (53 vs. 130), 2.9 times lower Zn/Se ratio (440 vs. 1290), 2 times lower Ca/Zn (1/7 vs. 1/3.5) and 1.2 times (8.2 vs. 10) lower Cu/Se ratio in PCa as compared to BPH. No significant differences (p values >0.05) for these ratios between PCa and BPH was observed in the present study. Among ratios, the most remarkable difference in the present study was a lower Zn/Fe ratio for PCa (vs. BPH) ($p = 0.04$). In the present study, somewhat higher Fe/Se and Ca/Se ($p = 0.068$ and 0.08 respectively) ratio in PCa (vs. BPH) could be noticed. So, there seems to be a relative deficiency of Zn and/or Se with regard to Fe in PCa tissue. Significance of this imbalance of these elements for prostate carcinogenesis needs further confirmation and exploration by larger studies.

There is scarcity of published data referring to inter-elemental correlations of various elements in normal, benign and malignant prostates [8, 9, 38]. Among Se, Zn and Fe, Zaichick and Zaichick [8] reported a positive trilateral correlation with each other in normal prostatic tissue, no correlation at all in BPH and a positive correlation of Zn with Se in PCa. However, in another study on prostatic tissues of healthy males, Zaichick [38] reported a significant positive correlation of the prostatic Zn with Ca, Cu, and Fe content and no correlation with Se. In our study, Zn was found to have positive correlations with Ca, Cu, Fe and Se in case of PCa and with Fe only in case of BPH. Se had a direct positive correlation with Zn ($r = 0.71$, $p = 0.02$) and Fe ($r = 0.77$, $p = 0.009$) in cancerous tissue. The positive correlation between Zn and Se in malignant tissue seems a consistent phenomenon as it was observed by Zaichick [8] as well. This interdependence between Se and Zn may partially explain the similar inconsistency of Se or Zn alone in preventing PCa. Fe had positive correlation with both Se and Zn in PCa as compared to BPH where it had positive correlation with Zn only. The significance of the noted positive correlation of Fe with Se in cancerous tissue needs further confirmation and exploration as this correlation ($r = 0.76$, $p = 0.009$) differed significantly (Z score = 2.15, $p = 0.03$) from an insignificant ($r = -0.13$, $p = 0.7$) correlation between them in BPH.

Thus, inability to retain high levels of Se and Zn in comparison to Fe may lead to imbalance between oxygen free radicals and antioxidative defense and thus possibly initiate and promote prostate carcinogenesis by oxidative DNA damage. Zn and Se deficiency may also lead to impaired immune function of cells. As Se and Zn supplementation alone have not resulted in consistent anticancer effects, targeting the intracellular Fe could be a potentially useful additional strategy. Recently, desferrioxamine by causing Fe depletion has been shown to suppress the critical oncogenic STAT3 (signal transducer and activator3) activity in vivo and vitro, on various cancer cell lines including those of prostate cancer [39].

Limitations of our study are small sample size and lack of normal prostate controls. Reasons for small sample size were; funding restrictions, the lesser prevalence of PCa in our geographical area, inclusion of the only samples where an adequate amount (at least 1 g) of representative tissue could be collected and inclusion of patients who didn't have any hormonal manipulation (like 5 alpha reductase inhibitors for BPH and hormonal ablation for PCa) prior to tissue sampling. Obtaining a normal prostate tissue from healthy age matched controls would have been an ethical issue for the study.

Despite these limitations, our study confirms the considerably divergent interrelationship of elements and their ratios in PCa versus BPH as noted by others as well [40].

Further understanding of such complex interrelationship of elements in carcinoma prostate may raise implications for anticancer strategies by either targeting the appropriate trace elements directly to produce an unfavourable environment of elements for carcinogenesis or exploiting these changes for new targeted therapies.

Conclusions

The malignant prostatic tissue has significantly lower zinc and selenium level, a lower Zn/Fe ratio and a positive direct correlation of Se with Zn and Fe as compared to benign prostatic hyperplasia tissue. Although importance of these findings either as a cause or effect of pathological process remains to be further investigated, understanding of such relative and differential elemental changes and their interdependence may be useful in defining the complex metabolic alterations in prostate carcinogenesis with potential for development of element based diagnostic, preventive or therapeutic strategies.

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Conflict of interest Bhupendra Pal Singh, Shailendra Dwivedi, Urmila Dhakad, Ramesh Chandra Murthy, Vimal Kumar Choubey, Apul Goel and Satya Narayan Sankhwar declare that they have no conflict of interest.

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Ethical standard All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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