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RACIAL AND ETHNIC VARIATION OF PSA IN GLOBAL POPULATION: AGE SPECIFIC REFERENCE INTERVALS FOR SERUM PROSTATE SPECIFIC ANTIGEN IN HEALTHY SOUTH INDIAN MALES

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ABSTRACT

The serum PSA is universally accepted as the useful and clinically relevant tumor marker for monitoring therapy and identifying early recurrence in patients of carcinoma prostate throughout the world. However, application of serum PSA is limited to screening for early adenocarcinoma prostate among males above fifty years of age.

Serum PSA concentration varies from one population to another in different parts of the world. Many groups of workers have selected 4 ng/ml of serum PSA as upper limit of normal range without giving due consideration for age specific increase in serum PSA. There is no single report available on normal decade wise age specific reference intervals for serum PSA in Indian males.

The present study is undertaken to establish age specific reference intervals in healthy Indian males from 20-89 years belonging to subpopulation of Andhra Pradesh from South India. Our results revealed lowest concentration of 95 percentile serum PSA in Indian males compared to other populations globally. Contrary to this, healthy Afro Americans were found to have highest concentration of serum PSA compared to all other populations.

KEY WORDS

Serum PSA, Healthy Indian males, reference intervals, Age specific PSA

INTRODUCTION

Prostate specific antigen is the useful and clinically relevant tumor marker for the diagnosis and management of prostate cancer. Unlike other tumor markers, prostate specific antigen (PSA) as the name indicates, is organ specific. PSA, synthesized by the epithelial cells lining the acini and ducts of the prostate gland is secreted via ductal system of prostate and stored in high concentration in seminal fluid. Under normal physiological condition it is present in lower concentration in circulation. With the help of sensitive enzyme immunoassays low levels of serum PSA are detected in healthy men of all races. Several studies have reported varying concentrations of PSA in serum of healthy males belonging to different races, ethnic groups and populations (1-4).

It is relevant to highlight that there is not a single report on either 95 percentile value (upper limit of normal range) or on appropriate reference intervals for serum PSA in Indian population. Hence it was felt mandatory to establish our own reference ranges, in particular, the 95 percentile for healthy Indian men in order to interpret the PSA results in benign as well as malignant disorders of prostate in Indian patients. Efforts were also made to establish age specific reference intervals for serum PSA concentrations because of the observation that it increases with advancing age in different populations (4-6)

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MATERIALS AND METHODS

Our study groups consisted of 583 healthy subjects (belonging to subpopulation of Andhra Pradesh from South India) of different age groups ranging from 19 years to 89 years. Subjects with any urological complications were excluded from the study. All the selected subjects underwent thorough clinical examination to exclude any other disorder or chronic illness. Blood samples were collected at room temperature by veiw puncture without anticoagulant. Serum was separated after complete clot retraction and stored at –20° C until further analysis.

PSA ESTIMATION

Serum PSA was estimated by one step double monoclonal antibody enzyme linked immuno-adsorbent assay (ELISA), using streptavidin coated tubes (Roche Enzymun-Test) by ELISA tube reader. The assay mixture contained 50 µl of calibrator or quality control serum or patient's serum, 1µl of the reagent

containing primary biotinylated monoclonal antibodies to PSA and secondary antibody conjugated to peroxidase. The reaction mixture was incubated for one and half hours at room temperature. The supernatant was discarded and the antigen-antibody complex immobilized on to the bottom of tube was washed thrice with wash solution to remove unbound antibodies. To this, 1µl of the substrate solution containing di-ammonium 2,2'-azino bis 3-ethylbenzothiazoline-6-sulphonate was added and the tube was incubated for one hour at room temperature. The intensity of bluish green color was measured at 405 nm. PSA concentration was directly proportional to the intensity of the color developed.

Statistical analysis was performed by student t test. Confidence intervals low-high mean, median, minimum and maximum were calculated for each decade age subgroups. Confidence interval low represented 5th percentile value and confidence interval high represented 95th percentile value in a given group.

RESULTS

The results of serum PSA concentrations in sera of 583 healthy males are presented in Table1. The maximum concentration of serum PSA in healthy males aged between 19 to 98 years was found to be 6.1ng/ml. The mean, median and 95 percentile PSA value for all age groups were 1.4ng/ml, 1.0ng/ml and 1.5ng/ml respectively. The PSA could not be detected in sera of 3.08% of healthy men in our study. PSA is more than 4 ng/ml in 14 persons out of 583 (2.4%).

The results on decade wise sub grouping of healthy men are presented in Table 1. The mean PSA value increased from 0.664 ng/ml for men younger than 30 years to 2.02 ng/ml for men older than 80 years. The 95 percentile value (the upper limit of normal range) rose from 1.07ng/ml for 20-29 years age group to 2.47 ng/ml for the older group (>80 years). The interesting thing to note was progressively significant increase in mean and median PSA concentration with advancing age right from 20 to 89 years. The maximum concentrations of serum PSA also increased progressively from1.7ng/ml to 6.1ng/ml for age groups 20-29 to 70-79 years. The maximum value of 5.0 ng/ml in men of more than 80 years was low compared to 70-79 years group. Contrary to this 95 percentile value was higher (2.47 ng/ml) in more than 80 years group compared to70-79 (2 0 ng/ml) years group.

DISCUSSION

Comparative data from world's literature on serum PSA reference values in healthy subjects of different populations have clearly shown slight but striking variations. Reports from different countries have documented serum PSA reference values either as minimum and maximum value or mean or 95 percentile (as upper limit of normal range) or a median value for their healthy subjects. In our study the results are presented as minimum, maximum, mean, median, lower and upper limit of 95 confidence intervals or percentile. Myrtel *et al* (3) found that 100% of healthy men less than 40 years of age and 97% of men 40 years or older had a serum PSA of below than or equal to 4.0 ng/ml. Of the 3% of men 40 years or older, none had a PSA value greater than 10 ng/ml. Investigators at John Hopkins hospital used mean ±3 standard deviations and reported upper limit of normal as 2.0 ng/ml for men less than 40 years of age and 2.8 ng/ml for men older than 40 years without any prostatic disease (1,7). Stamey *et al* (8) and Yang (9) established upper limit of reference ranges as 2.5 ng/ml (mean±2S.D) for 157 normal men (21 to 76 years) without any existing prostatic problem including BPH. An international multicentre study coordinated by M/s Boerhinger Mannheim from Germany in 1996 (10) covered more than 70 centres including ours from18 countries (Austria, Canada, Czechin, France, Germany, India, Israel, Italy, Japan, KSA-Saudi Arabia, Netherlands, New Zealand, Poland, Portugal, South Africa, Spain, Sweden, Turkey and UK). The estimation of serum PSA in 1063 asymptomatic healthy men revealed a minimum serum PSA value as 0.001 and maximum as 14.8 ng/ml. The median total PSA concentration for all ages was 0.77 ng/ml. Surprisingly this study did not document mean or 95 percentile value. While carrying out multicentre multidisciplinary project of American Cancer Society National Cancer Detection programme, Mettlin *et al* (11) observed mean serum PSA as 2.1ng/ml on 2011 healthy American white participants with mean average age of 64.5±5.0 years. The average median PSA concentration for all age groups in our study was 1.0 ng/ml for 583 healthy men contrary to BM multicentre study.

Enormous efforts were made from 1995 onwards to establish age specific reference ranges for serum PSA throughout the world in order to find out serum PSA variation in different populations. Extensive work by Osterling group (4,12,13) on American whites and Japanese men clearly showed age specific significant increase for 95 percentile (upper limit of normal range of serum PSA) with advancing age (4,12). These results clearly show that one cannot rely on a single reference for all age groups and hence emphasis was made to establish age specific reference ranges for different populations (Table 2). Oesterling(12) showed that 95 percentile values in healthy Japanese from small fishing village of Shimamaki-mura, Japan were comparatively lower in all decade wise age subgroups compared to PSA concentration in American white men. Crawford *et al* (6) observed similar results on American whites during the prostate cancer awareness weak. Further studies by Catalona and Weinrich (14,15) revealed more or less similar results on US white population. From these reports it is evident that the 95

percentile, the upper limit of normal PSA correlated well with advancing age. The published reports (15,16) have shown higher 95 percentile i.e., the upper limit of normal range in American blacks compared to American white men. The serum PSA concentrations were lower in Japanese, Chinese, Indians and in Singaporeans compared to Americans (12,17-19). Interestingly Indian males had very low concentrations of PSA compared to healthy males from other Asia pacific countries. The lower limit of normal range in all populations did not show any value as in many subjects the PSA could not be detected by a given immunoassay method. However the current third generation immunoassays based on chemiluminescence technology are able to detect today a minimum concentration 0.006 ng/ml in healthy sera. Minimum value of PSA is for normal reference intervals shown as zero throughout the globe in many reports. Healthy men from and US and Turkey had higher mean serum PSA compared to many other populations in all age groups (20-22,6). Our results for mean serum PSA in healthy Indian males were slightly higher compared to men from Korea, but slightly lower than American men at different age groups relatively.

During health fair of the midlands in US it was noted that 1716 American white men residing in Nebraska, U.S. (22) had slightly variable 95 percentile PSA concentrations compared to American white men residing in other parts of U.S. (22). The observations made in a community based study during prostate cancer awareness week during 1993 and 1994 revealed similar mean serum PSA concentrations in Indians and American white men (Table 2) residing in Denver USA in the 50-59 and 60-69 years age groups (23). The 95 percentile serum PSA concentrations reported by Lin *et al* (17) and Kao *et al* (18) for Taiwanese and Chinese have shown higher values compared to Singaporean men of all age groups except for men between 70-79 years. Korean and Singaporean men have shown similar 95 percentile PSA values for all age groups (19,20,24). Two centers have reported significantly higher concentration of serum PSA in American blacks compared to American whites (15,16).

The cut off value of 4 ng/ml as upper limit of normal reference interval has been widely used by many groups, but this value does not take into account the age related increase in serum PSA. Our results on Indian men substantiated that there is an increase of PSA with advancing age. Hence the routine use of age specific reference intervals for PSA will improve the diagnostic efficiency in prostate cancer patients from India and will help in segregating individuals carrying high risk of harboring very early focus of prostate malignancy.

In summary all the groups of workers including U.S. have shown age specific significant increase of serum PSA concentration variability among different ethnic and racial groups. These age specific reference ranges have the potential to make serum PSA a mere discriminating tumor marker for men and sensitive tumor marker for men below 60 years of age and a more specific tumor marker for men above 60 years of age. Additionally age specific serum PSA will help in developing specific algorithms for different populations to detect non-palpable adenocarcinoma of prostate at very early stage. This could be achieved by serial determinations of serum PSA at appropriate intervals if the individual had serum PSA concentration beyond 95percentile value specific for decade wise age subgroups.

Table 1: Age Specific PSA in Healthy Males

PSA (ng/ml)	20-29 (A)	30-39 (B)	40-49 (C)	50-59 (D)	60-69 (E)	70-79 (F)	80-89 (G)	All ages
N	40	52	58	148	164	86	35	583
Mean ±SD	0.664 ±0.41	0.972 ±0.516	1.14 ±0.76	1.31 ±1.074	1.414 ±1.23	1.67 ±1.497	2.02 ±1.19	1.4 ±1.24
Median	0.8	0.9	1.2	1.0	1.0	1.23	2.2	1.0
SEM	0.11	0.07	0.102	0.09	0.096	0.165	0.205	0.056
Lower 95% CI	0.6	0.84	0.98	1.13	1.23	1.35	1.64	1.3
Upper **95%CI**	1.07	1.13	1.39	1.48	1.609	2.0	2.47	1.51
Min-Max	0-1.7	0-2.2	0-2.9	0-4.8	0-5.5	0-6.1	0-5.05	0-6.1
P		A vs B 0.0687	B vs C 0.0028	C vs D 0.0017	D vs E 0.047	E vs F 0.0166	F vs G 0.0674	-

Table 2: Age Specific Serum PSA (ng/ml) in Different Populations

Author (Year, n)	Population	< 40 (yrs)	40-49 (yrs)	50-59 (yrs)	60-69 (yrs)	70-79 (yrs)	>80 (yrs)
PSA 95 PERCENTILE VALUE							
Oesterling et al (1987)	Caucasians	-	2.5	-	-	6.5	-
Oesterling et al (1993,n=471)	Whites	-	2.5	3.5	4.5	6.5	-
Dalkin et al (1993,n=728)	US (Tuscan)	-		3.5	5.4	6.3	-
Anderson et al (1995,n=1716)	US Nebraska	-	1.5	2.6	4.4	7.5	-
Weinrich et al (1998,n=1319)	US Blacks US Whites	- -	1.9 -	3.8 2.7	5.7 4.9	- -	- -
Cooney et al (2001,n=432)	Afro-Americans	-		2.36	-	-	5.59
Morgan et al (1996,n=B-1673 W-1802)	AfroAmericans US Whites	- -	2.4 2.1	6.54 3.6	11.3 4.3	12.5 5.8	- -
Lee et al (2000,n=5805)	Korean	-	2.0	2.4	3.9	6.3	-
Oesterling et al (1995,n=286)	Japanese	-	2	3	4	5.0	
Lin et al (1996,n=1008)	Chinese (Taiwan)	1.9	2.59	3.31	5.03	5.73	-
Kao et al (1997,n=414)	Chinese	1.5	1.88	2.37	4.82	5.86	
Saw et al (2000)	Singaporean	1.4	1.7	2.3	4.0	6.3	6.6
Our Study (2002,n=583)	Indians	0.9	1.3	1.48	1.6	2.0	2.47
PSA MEAN VALUES							
Stamey et al (1987,n=157)	American	1.2	1.19	1.2	1.15	-	-
Yang et al (1987)	American	0.95	1.19	1.2	1.15	-	-
Dalkin et al (1993,n=728)	US (Tuscan)	-	-	1.32 ±1.1	1.91 ±1.72	2.36 ±1.98	-
Dalkin et al (1995,n=5469)	US Multicenter	-	-	3.9	-	7.6	-
Atalay et al (1998)	Turkey	-	1.7	2.0	2.9	3.5	-
Crawford et al (1993)	US	-	1.5	1.6	3.1	3.3	-
Anderson et al (1995,n=1716)	US	-	1.5	2.5	4.5	7.5	-
De Antoni et al (1996)	US (Denver)	-	0.79	1.33	1.94	2.35	-
BM Intl.Multi Center study (1996,n=1032)	Global	0.73	0.73	1.0	1.35	1.63	-
Lee et al (2000,n=1008)	Korean	0.8	0.8	0.9	1.0	1.3	-
Our Study (2002,n=546)	Indian	0.826 ±0.5	1.14 ±0.76	1.31 ±1.07	1.413 ±1.23	1.68 ±1.5	2.01 ±1.19

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