

ROLE OF FREE / TOTAL PSA RATIO TO DIFFERENTIATE BPH AND PROSTATE CANCER

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ABSTRACT

This study was undertaken to determine Free to Total PSA ratio to differentiate between Benign Prostate Hyperplasia (BPH) and Prostate Cancer (PCa) and to improve the accuracy of the PSA test and the specificity of PCa detection particularly when PSA levels fall between 4-10ng/ml levels. The study group consisted of a total of 124 subjects. Free PSA and Total PSA were determined using assay, which is based on Chemiluminescent Microparticle Immunoassay (CMIA) technology. The Statistical test used were Students' t test for difference of Mean and Pearson Correlation test, using SPSS and MS Excel Statistical packages. Free/Total PSA ratios in the prostate cancer group were significantly lower than those in the BPH group. A cut-off of F/T PSA ratio% at 18% improves diagnostic sensitivity and specificity for prostate cancer.

KEYWORDS: Benign Prostate Hyperplasia (BPH), Prostate cancer (Pca), Free PSA, Total PSA, Chemiluminescent Microparticle Immunoassay (CMIA)

PCa is the third most common cause of death from cancer in men of all ages. It is the most prevalent cancer found in men above the age of fifty year and is frequently diagnosed in men (Malati and Rajanikumar, 2006) between 45 and 90 years of age with a median age of 72 years. The age of Indian patients of PCa varies from 35-86, which is much lower when compared with average age of patients in western countries. The actual incidence of prostate cancer in India and in nonresident Indians is lower than that in the Western populations (Krishnamoorthy and Padmanabha, 2016). According to a recent study, PCa ranks fifth in incidence and fourth in cancer mortality for men in Mumbai and the incidence is increasing by 1% every year (Yeole, 2008). Fifty to sixty percent of men over the age of 60 in India have Benign Prostate hyperplasia (BPH). 30-40% show related symptoms and 10% out of this group may have PCa. BPH affects 40 percent of men in their 50s and 90 percent of men in their 80s. BPH is a noncancerous enlargement of the prostate gland that may restrict the flow of urine from the bladder. The possibility of having PCa varies with age, being 13.7% for the age group 60-79 years, 2.2% for those aged 40-59, and 0.005% in those younger than 39 years. Men who have a brother or father with PCa have twice the usual risk of developing PCa (Steinberg *et al.*, 1990). Gene alterations on chromosome 1, 17, and the X chromosome have been found in some patients with a family history of PCa. African American men have a higher prevalence and more aggressive prostate cancer than white men, who, in turn, have a higher prevalence than men of Asian origin (Hoffman *et al.*, 2001). Men with higher serum

levels of the short-chain omega-6 fatty acid linoleic acid have higher rates of PCa. However, the same series of studies showed that men with elevated levels of long-chain omega-3 fatty acids (EPA and DHA), found in fatty fishes like salmon had lowered incidence (Gann and Giovannucci, 2005). Other dietary factors that may increase PCa risk include low intake of vitamin E and the mineral selenium. Decreased levels of vitamin A may be a risk factor because this can promote cell differentiation and stimulate the immune system. Vitamin D deficiency was suggested as a risk factor, and studies show an inverse relationship between ultraviolet exposure and mortality rates for PCa (Peters *et al.*, 2007).

PCa can also be attributed to hormonal reasons. For instance, androgen ablation causes a regression of PCa. Obesity and elevated blood levels of testosterone might increase the risk for PCa. Daily use of anti-inflammatory medicines such as aspirin, ibuprofen, or naproxen may decrease PCa risk. Use of the cholesterol-lowering drugs known as the statins may also decrease PCa risk (Hsing and Comstock, 2009) (Jacobs *et al.*, 2005) (Shannon *et al.*, 2005) (Platz *et al.*, 2005).

More frequent ejaculation also may decrease a man's risk of PCa (Giles *et al.*, 2003). The researchers hypothesize that this could be because regular ejaculation reduces the buildup of carcinogenic deposits such as 3-methylcholanthrene, produced from the breakdown of cholesterol, which could damage the cells lining the prostate.

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Symptoms for PCa

Early diagnosis of PCa is hindered by lack of symptoms in men with localized tumors. Some men, however, will experience symptoms such as frequent, hesitant, or burning urination, difficulty in having an erection, or pain or stiffness in the back, hips or upper thighs. Some advanced PCa can slow or weaken the urinary stream or increase the need to urinate more often. But noncancerous diseases of the prostate, such as BPH are a more common cause of these symptoms. If the PCa is advanced, one might develop blood in the urine (hematuria) or might find it difficult to get an erection (impotence). Advanced PCa commonly spreads to the bones, which can cause pain in the hips, spine, ribs, or other areas (Springhouse, 2005). Cancer that has spread to the spine can also cause it to press on the spinal nerves, which can result in weakness or numbness in the legs or feet, or even loss of bladder or bowel control.

Diagnosis of Prostate Cancer

The following screening tests may be used initially.

1. Digital rectal examination (DRE): Data from community-based studies suggest that the positive predictive value of digital rectal examination for prostate cancer is 15% to 30% and varies relatively little with age (Chodak *et al.*, 1989).
2. Prostate Specific Antigen (PSA) Test: If higher than normal levels of PSA are detected or if levels rise over time, it could indicate prostate inflammation, prostate enlargement or prostate cancer (Brawer *et al.*, 1992).
3. Ultrasound (Transrectal Ultrasonography): A transrectal ultrasonography (TRUS) provides a visual image of the prostate and is used if the DRE indicates the presence of cancer, the procedure may help to confirm an uncertain preliminary diagnosis and is useful as a guide for needle biopsies (Gupta *et al.*, 2005).
4. Biopsy: This is more of a confirmation diagnosis.

These tests are also used to determine which tumors are localized within the prostate and are, thus, potentially treatable. Observational studies suggest that DRE and PSA, combined with TRUS and biopsy, can identify localized prostate cancer in 3-5% of men, although

the tests do result in a number of false positives and negatives.

Treatment for Prostate Cancer

Three major treatment options are available for localized prostate cancer: radical prostatectomy, radical radiotherapy and conservative management (involving monitoring and treatment of symptoms) (Milin, 1947).

PSA

PSA has been characterized as the most useful serum marker for the detection and management of PCa, however, Screening with PSA is problematic because it is really an organ specific marker for the prostate rather than a specific marker for cancer. There is considerable overlap in PSA concentration in men with benign prostate disease. In this regard, the recognition of the molecular forms of PSA, Free PSA has shown the most promise. Although PSA is considered to be the most effective tumor marker in human oncology, its role in screening strategies for PCa has not been completely established. It is a glycoprotein produced exclusively by the epithelial cells lining the prostatic ducts and acini. Normally, it is secreted into the prostatic ducts and is present only in prostate tissue, prostatic fluid, and seminal plasma. PSA is produced by normal, hyperplastic, and cancerous prostatic tissue (Emil and Jack). A major function of PSA is proteolytic cleavage of gel forming proteins in the seminal fluid, resulting in the liquification of the seminal gel and increased sperm mobility.

PSA and Protease Inhibitors

PSA circulates in serum in complex forms (bound to protease inhibitors) or in non-complex (free or unbound) forms. Approximately 60% to 70% of PSA occurs in an active free form (Zhang *et al.*, 1998) with less than 5% in complex with protease inhibitors. The major protease inhibitor, to which active single-chain PSA is covalently linked in a 1:1 molar ratio, is PCI where by the PSA-PCI complex could only be detected in seminal plasma and not in serum.

PSA and Protease Inhibitors in Blood

PSA occurs in three major forms in the blood. The major immunodetectable form is PSA complexed with the serine protease inhibitor and alpha-1-antichymotrypsin (PSA-ACT). In contrast to seminal fluid, 55% to 95% of immunodetectable PSA forms covalent 1:1 molar

complexes with ACT. Uncomplexed, or FREE PSA, is the other immunodetectable form of PSA in serum between 5% to 45% of the immunodetectable PSA is found in a free noncomplexed form, which is most likely catalytically inactive as it remains virtually nonreactive with the large excess of protease inhibitors (ACT or AMG) in blood. A third form of PSA, a complex with alpha-2-macroglobulin (AMG) is not detectable with current immunoassays for PSA due to the engulfment and subsequent masking of PSA epitopes by the alpha-2-macroglobulin molecule (Stephan et al., 1981). The serum concentration of the PSAAMG complex is difficult to assess, likely due to the conformation of the AMG molecule that engulfs the PSA molecule, hence blocking antibody access to the PSA epitopes.

PSA level in the blood is found if the barrier between the epithelium and the blood stream is damaged. Typical sources for damage are cancer, bacterial infection, and prostate infection or destruction of part of the prostate by damage to its blood supply. PSA is also present in urine and serum. Low levels of PSA are found in the blood as a result of leakage of PSA from the prostate gland. Increasing levels of PSA are associated with prostatic pathology; including prostatitis, BPH, and PCa (Lija *et al.*, 1999). Numerous studies have shown that for groups of men with PCa, serum PSA correlates directly with pathologic stage. However, in most cases, PSA level alone does not provide accurate information because of overlap in PSA level between stages. False positive elevations in the PSA increases in the PSA that are caused by conditions other than PCa for example BPH and infection or prostates can elevate the PSA (Ornstein *et al.*, 1998).

Role of PSA in Pca

In general, normal PSA levels are below 4ng/ml. A reading between 4 and 10ng/ml indicates about 20% chance that cancer is present and a measurement above 10ng/ml is considered a strong indication of PCa (Catalona and Scoott, 1986). But because various factors can cause PSA levels to fluctuate, one abnormal PSA test does not necessarily indicate that cancer is present. Once cancer is diagnosed, PSA level is also measured to determine its extent. If PSA level is less than 20ng/ml then it is likely the cancer has not spread. Metastasis is strongly indicated as the level rises over 40ng/ml. PSA level is also used for monitoring the

course of the disease after initial treatment of prostatic cancer. Rising level indicates recurrence of tumour, although the exact location cannot be determined just by using PSA. In contrast to this, if the PSA falls significantly after treatment and remains persistently low, then it will indicate the disease is under control (Lodding *et al.*, 1998).

The present study is undertaken with a view to determine Free to Total PSA ratio to differentiate between BPH and PCa and to improve the accuracy of the PSA test and the specificity of PCa detection particularly when PSA levels fall between 4-10ng/ml levels.

MATERIALS AND METHODS

- Target population was male referred to genitourinary unit of our Institute between the age group 50–90 yrs. In the duration of course the medical records of histological proven patients were studied. The blood sample were collected from these patients. Normal control blood were taken from 30 healthy ambulant donors. Serum were extracted by centrifugation of samples at 4000rpm for 10 min, and were stored at –20 °C until use.

Methods

- Serum levels of Free PSA and Total PSA were determined using assay, which is based on Chemiluminescent Microparticle Immunoassay (CMIA, Marketed) technology. The Architect i system and reagent kit from Abbott were used.
- The Statistical test used were Students' t test for difference of Mean and Pearson Correlation test, using SPSS and MS Excel Statistical packages.

RESULTS AND DISCUSSION

The study group consisted of 104 male patients comprising of 54 (57%) PCa patients and 40 (43%) patients with BPH. The mean age for these groups was 65.90 ± 8.11 and 67.32 ± 7.54 respectively. 30 age matched healthy male formed the control group with the mean age of 60.90 ± 7.17 . For the three groups viz. Normal cases, BPH patients and PCa patients, the data was analyzed for FPSA, TPSA and F/T PSA%. Following is the tabulation for the data.

Table 1: Normal cases, BPH patients and PCa patients

| Normal Healthy controls | | | |
|-----------------------------|----------------|-----------------|-----------------|
| | FPSA ng/ml | TPSA ng/ml | (FPSA)/(TPSA) % |
| Range | 0.040 to 1.162 | 0.147 to 4.00 | 25.98 - 58.04 |
| Mean | 0.38 | 1.12 | 37.17 |
| Standard Deviation | 0.26 | 0.68 | 10.66 |
| Benign Prostate Hyperplasia | | | |
| | FPSA ng/ml | TPSA ng/ml | (FPSA)/(TPSA) % |
| Range | 0.386 to 3.396 | 2.075 to 16.816 | 10.47 -54.6 |
| Mean | 1.75 | 7.54 | 24.68 |
| Standard Deviation | 1.02 | 4.64 | 10.56 |
| Prostate Cancer | | | |
| | FPSA ng/ml | TPSA ng/ml | (FPSA)/(TPSA) % |
| Range | 0.052 to 2.937 | 2.078 to 30.0 | 4.06 - 20.85 |
| Mean | 1.02 | 9.29 | 10.91 |
| Standard Deviation | 0.94 | 7.82 | 6.92 |

We note that the mean TPSA levels are the least for the healthy patients and it increases in diseased condition being higher in prostate cancer patient. We also note that the F/T PSA % ratio were significantly different amongst the three groups that we have at a significance level ($p < 0.001$). Percentage F/T PSA levels ranged from 10.47 to 54.6 % (mean \pm SE, 24.68 ± 10.56) and from 4.06 to 20.85% (mean \pm SE, 10.9 ± 6.92) in patients with BPH and PCa, respectively. The mean F/T PSA ratio was found to be significantly lower in the group of patients with prostate cancer. This finding is consistent with the findings of Filella *et al.*, (1997).

Our findings are consistent with the study done by V Thakur *et al.* wherein they discussed the utility of F/T PSA ratio in diagnosis of prostate carcinoma. They found that mean total PSA was the least in normal healthy subjects and was the highest in the cancer patients. Further they also found that the F/TPSA ratio was significantly different between the three groups. Recently numerous studies have demonstrated a lower ratio of FPSA/TPSA in Ca P patients compared with patients with BPH (Brawer *et al.*, 1992) (Gupta *et al.*, 2005) (Milin, 1947)

If we look at the frequency distribution of no. of cases of Normal, BPH and Cancer across various values of Total PSA, as shown in the graph below:

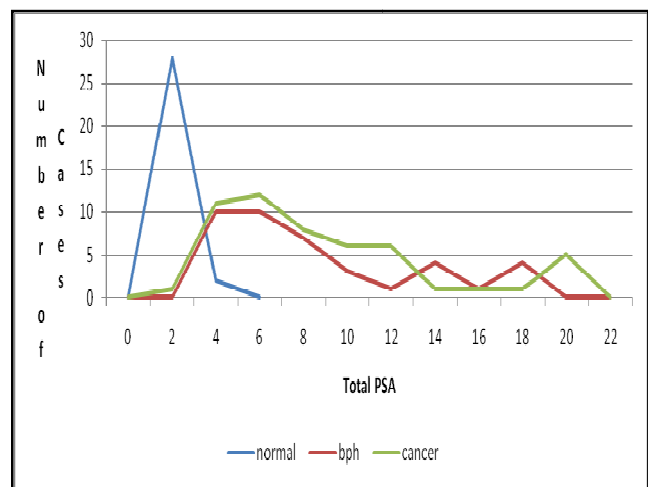


Figure 1: BPH and Cancer across various values of Total PSA

We find that there is an area of overlap between BPH and Cancer between the Total PSA values of 4 to 10; *the grey zone*. Theoretically, the occurrence of the various PSA forms in serum allows for a better discrimination between prostate cancer and BPH (Malati and Rajanikumar, 2006).

If we do the two tailed t-test for difference in mean between Cancer, BPH and control (normal) we find that there is significant difference between BPH (24.68 ± 10.56) and control (normal) (37.17 ± 10.66 , $p = 0.00$) and between Cancer (10.91 ± 6.92) and control (normal) (37.17 ± 10.66 ,

p= 0.00) (significance level $\alpha=0.05$, using student’s t test for difference of means)

Now if we replicate a similar test on the Cancer and BPH groups to find if there is a significant difference between the mean of two groups we find that they are significantly different with the value of p =0.000 at a significance level of $\alpha=0.05$. This establishes that the ratio (fPSA/TPSA) % can be used to identify cases of Cancer from BPH in the grey zone (TPSA from 4 to 10ng/ml)

The study was performed on 94 consecutive male patients consisting of 40 patients with BPH and 54 PCa patients. The sensitivity and specificity was calculated at different %fPSA/TPSA ratio cutoff. From cutoff value 16 to 22, sensitivity increased from 86% to 96%, but specificity decreased from 98% to 80%. Increasing the cutoff value thereafter showed no change in sensitivity, but specificity further reduced to 65%. Hence in this patient’s series we find that at 18% (%fPSA/TPSA) we get the maximum sensitivity with a stable level of specificity.

Also if we look at plot of positive predictive and negative predictive value vs %fPSA/TPSA we find that at 18% point we have a very high level of “positive predictive value” and a relatively high and stable level of “negative predictive value”. The trend is evident even in the plot of “False negative” and “False positive” vs %fPSA/TPSA plot. Here again we see that at 18% point, the “False negative” is the lowest with a relatively very low and stable level of “False positive” cases

Hence we can see that 18% ratio fTPSA/TPSA can be taken as a cutoff value to differentiate between BPH and PCa cases; any value of the % ratio fPSA/TPSA lower than 18% is indicative of a PCa case and any value higher than 18% is indicative of BPH.

The cutoff found here is similar to the findings of a study conducted in Shaikh Zayed hospital & Mayo hospital by Saima Naz, Sarah Ahmad *et al.*, wherein they found that at the 18% cutoff point, the sensitivity was 86% and specificity was 94%. Using a cutoff of 18%, the free-PSA test significantly improved the differential diagnosis of BPH and Ca P in the ‘gray zone’ as compared to the use of total PSA alone in the study group.

In a case controlled study of 200 patients conducted by Iqbal *et al.*, at Jinnah hospital, Lahore, found that at 18% cutoff, the test gives a specificity and sensitivity

of 91.9% and 92.1% respectively with 87.2% PPV. Further concurrence on 18% cutoff for the test was found with a study done by Gaspar and Arribas in 2000, Madrid hospital.

Numerous retrospective studies demonstrated that ~ 19–64%of unnecessary, negative biopsies could be eliminated (Jacobs *et al.*, 2005) (Brawer *et al.*, 1992) (Stephan *et al.*, 1981) Reasons for these substantial differences could be the use of a wide range of fPSA% cutoffs (14–28%) with different assay.

Currently there are many different recommendations and different cutoffs for the use of fPSA% depending on the various assays used and on the targeted sensitivity or specificity, the reported rates of sensitivity, specificity, and predictive values are widely scattered (Chodak *et al.*, 1989) (Brawer *et al.*, 1992). The analytical variability of assay methods could be the cause of discrepancies among the results of different studies (Milin, 1947). But, let us evaluate the strength of the test based on the %fPSA/TPSA with cut off 18% on the patient data that we have. (Table 2)

Table 2: Percentage of fPSA/TPSA

| TPSA>=4 | BPH | Cancer | Total |
|-------------------------|-----------------------|-----------------------|-------|
| Actual | 30 | 41 | 71 |
| TPSA | 0 | 71 | 71 |
| %fPSA/TPSA (18%) | 28 | 43 | 71 |
| | False negative | False positive | |
| TPSA (>=4) | 0 | 30 | |
| %fPSA/TPSA (18%) | 0 | 2 | |

If we first apply the TPSA test, then all the cases where TPSA>=4, 71 out of 124, will be indicative for biopsy. From the actual data, we know that only 41 out of these 71 are actual positive cancer cases. This means that 30 cases of those sent for biopsy are only BPH and negative for PCa.

Now, if we apply our %fPSA/TPSA ratio test with a cutoff of 18%, then only those cases (amongst those with TPSA>=4) where the ratio<=18% will be sent for biopsy i.e. 43. Comparing it with actual data we find that of the 43 cases sent for biopsy only 2 cases are those which are only BPH and negative for prostate cancer.

Comparing 30 false positive cases versus only 2 false positive cases, we can easily conclude that employing the ratio test along with the TPSA test increases the effectiveness of differentiating cases of BPH from positive cancer cases. We further try the ratio test with 18% cutoff on the “Grey zone” or overlap between BPH and Cancer cases which is between $4 \leq \text{TPSA} < 10$ and we have the following results (Table 3)

Now, if we apply our %FPSA/TPSA ratio test with a cutoff of 18% to the grey zone (TPSA 4- 10ng/ml)

Table 3: TPSA Test

| $4 \leq \text{TPSA} < 10$ | BPH | Cancer | Total |
|---------------------------|-----------------------|-----------------------|-------|
| Actual | 20 | 26 | 46 |
| TPSA | | 46 | 46 |
| %FPSA/TPSA (18%) | 19 | 27 | 46 |
| | False negative | False positive | |
| TPSA (≥ 4) | 0 | 20 | |
| %FPSA/TPSA (18%) | 0 | 1 | |

In the above tables we can easily see only a TPSA test is unable to differentiate between cases of BPH and Cancer. But if we apply the ratio test with cutoff of 18% we find that, it detects 19 BPH cases compared to 20 as per actual data and 27 cancer cases compared to 26 as per actual data. So we can conclude that the %ratio FPSA/TPSA test, with a cutoff of 18%, is highly effective at differentiating BPH cases from cancer cases in the “grey zone”.

We found that the percent free PSA was not associated with patientage, in agreement with Oesterling *et al.*, Filella *et al.*, Morgan *et al.*, and Jung *et al.* A recent, prospective study by Catalona *et al.*, showed a statistically significant and clinically relevant direct association between percent free PSA and age. (Table 4)

Now, if we apply our %FPSA/TPSA ratio test with a cutoff of 18% to the cases where $\text{TPSA} \leq 4 \text{ng/ml}$

Table 4: Percentage FPSA/TPSA ratio test

| TPSA < 4 | Non Cancer | Cancer | Total |
|-------------------|-----------------------|-----------------------|-------|
| Actual | 40 | 12 | 52 |
| TPSA | 52 | | 52 |
| %FPSA/TPSA (18%) | 40 | 12 | 52 |
| | False negative | False positive | |
| TPSA (≥ 4) | 12 | 0 | |
| %FPSA/TPSA (18%) | 0 | 0 | |

For the cases where $\text{TPSA} < 4$, if we use only TPSA as a test, all the cases would be diagnosed as negative for cancer and none would be sent for biopsy as a result. From the actual data we find that this would be a wrong diagnosis for 12 of the cases, which should have been sent for Biopsy. However, if we use %FPSA/TPSA test with a cut off of 18%, as found earlier, we would be correctly identifying all the 12 cases as positive for cancer and would be rightly sending them for biopsy.

Hence we see that for cases where $\text{TPSA} < 4$, the test %FPSA/TPSA enhances the sensitivity of detecting the cancer patients. Needless to say it will help in early detection for cancer.

CONCLUSION

In conclusion, F/T PSA ratios in the PCa group were significantly lower than those in the BPH. A cut-off of F/T PSA ratio% at 18% improves diagnostic sensitivity and specificity for PCa.

The results from this study show that the use of the percentage FPSA value ratio improves PSA-based differential diagnosis of PCa in patients and help differentiate PCa from BPH and reduce unnecessary prostate biopsies. Selective measurement of percent free PSA in cases of uncertain diagnosis can improve the specificity of PCa detection especially when the TPSA falls between 4-10ng/ml thus making F/T PSA ratio an excellent adjuvant to TPSA for diagnosis of PCa, increasing the specificity and predictive value for positive test which in turn will assist the physician in determining the probability of PCa, managing the patient and taking informed care

decision. In the near future, the FPSA% value will be used more frequently with additional clinical and laboratory values to calculate the risk of having cancer.

In conclusion, we predict that better insight in regard to PSA molecular forms will lead not only to better diagnostic markers, but also to an understanding of PCa development and possible new therapeutic options.

FPSA measurements and the use of its ratio to TPSA are well established in numerous, large-scale, multi-institutional clinical trials. A forthcoming standardization of the FPSA and TPSA measurements will provide a better comparability of different PSA assays. In the near future, the FPSA% value will be used more frequently with additional clinical and laboratory values to calculate the risk of having cancer.

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COMPARISON OF MAXIMAL O₂ UPTAKE IN AMATEUR & ELITE BADMINTON PLAYERS

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ABSTRACT

Badminton is a racquet sport played using racquets to hit a shuttlecock across a net¹. Competitive matches last between 40 minutes and an hour, at a high intensity; intense rallies usually go for six to eight seconds. VO₂ max is a measure of maximum rate of oxygen consumption measured during incremental exercise (exercise of increasing intensity), which is an important factor in elite players since people playing at different levels have different set of physiological pre-requisites for the game. Hence, our study aims to compare the maximal O₂ uptake in amateur and elite badminton players. This analytical study was conducted in Amateur and elite badminton players between the age group of 10 to 20 years. A total of 50 players (25 elite and 25 amateur) were included in the study. The Yo-Yo intermittent recovery test, level 1, was used for estimation of VO₂ max. An analysis of the data showed that elite badminton players had a significantly higher VO₂ max as compared to amateur players. Hence, the study concluded that VO₂ max consumption is higher in elite players and requires to be enhanced in amateur players for them to be able to continue their play effortlessly for a longer period of time.

KEYWORDS: Badminton Players, VO₂ max, Maximal O₂ Uptake, YO-YO Test

Badminton is a racquet sport played using racquets to hit a shuttlecock across a net. Although it may be played with larger teams, the most common forms of the game are "singles" (with one player per side) and "doubles" (with two players per side). Badminton is often played as a casual outdoor activity in a yard or on a beach; formal games are played on a rectangular indoor court. Points are scored by striking the shuttlecock with the racquet and landing it within the opposing side's half of the court. (Bisi *et al.*, 2011)

To win a game, a player or pair needs to score 21 points. Points are scored by one player or pair after every rally. Competitive matches last between 40 minutes and an hour, at a high intensity; intense rallies usually go for six to eight seconds. Although a badminton match goes for less than half the time of a typical tennis match (between two hours and 45 minutes and three hours for tennis), badminton players tend to run twice as far and hit nearly twice as many shots (badminton players run around 6.4km and tennis players around 3.2km).

Maximal oxygen consumption (VO₂ max) is defined as the ability to transport and consume oxygen

during exhausted work and is related to cardiorespiratory fitness. (Astorino *et al.*, 2005)

In the exercising human, maximal oxygen uptake (VO₂ max) is limited by the ability of the cardiorespiratory system to deliver oxygen to the exercising muscles. This is shown by three major lines of evidence: 1) when oxygen delivery is altered (by blood doping, hypoxia, or beta-blockade), VO₂ max changes accordingly; 2) the increase in VO₂ max with training results primarily from an increase in maximal cardiac output (not an increase in the a-v O₂ difference); and 3) when a small muscle mass is overly perfused during exercise, it has an extremely high capacity for consuming oxygen. Thus, O₂ delivery, not skeletal muscle O₂ extraction, is viewed as the primary limiting factor for VO₂ max in exercising humans. Metabolic adaptations in skeletal muscle are, however, critical for improving submaximal endurance performance. Endurance training causes an increase in mitochondrial enzyme activities, which improves performance by enhancing fat oxidation and decreasing lactic acid accumulation at a given VO₂. VO₂ max is an important variable that sets the upper limit for endurance performance (an athlete cannot operate above 100% VO₂ max, for extended periods) (George *et al.*,

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2009). A few studies have evaluated the physiological demands of competitive badminton players. Faccini and Dal Monte (1996) studied elite badminton players from Italy and found the mean and maximum VO₂ during match play to be 35±4 ml/kg/min and 51.8±5.8 ml/kg/min.

The American College of Sports Medicine (ACSM) has published several metabolic equations for the indirect estimation of VO₂ max while walking, running, and stepping as well as for leg and arm ergometers. (Lee *et al.*, 2011)

In the laboratory setting, the most accurate way to assess VO₂ max is undoubtedly via applying a maximal graded exercise test (GXT) performed to volitional exhaustion on a motorized treadmill or cycle ergometer while expired air is analysed continuously by gas analysers (Krustrup *et al.*, 2003) (Bangsbo *et al.*, 2008). This is the direct method of measuring VO₂ max. However, equipment costs and staff training limit direct measurement mainly to research and few clinical settings. Hence, in this study, we have used an indirect method of measuring VO₂ max which is the yo-yo intermittent recovery test.

There are two Yo-Yo intermittent recovery (YYIR) tests namely YYIR1 and YYIR2. These evaluate an individual's ability to repeatedly perform intense exercise. The Yo-Yo IR level 1 (Yo-Yo IR1) test focuses on the capacity to carry out intermittent exercise leading to a maximal activation of the aerobic system, whereas Yo-Yo IR level 2 (Yo-Yo IR2) determines an individual's ability to recover from repeated exercise with a high contribution from the anaerobic system. The Yo-Yo IR tests provide a simple and valid way to obtain important information of an individual's capacity to perform repeated intense exercise and to examine changes in performance.

In this study, amateur players are the players who have been practicing badminton for less than 1 year and elite players are the ones who are playing at competitive levels like district, state or national level. Now as we know, VO₂ max is a measure of maximum rate of oxygen consumption measured during incremental exercise (exercise of increasing intensity), which is an important factor in elite players since people playing at different levels have different set of physiological pre-requisites for the game (Lieshout, 2002). Hence this study was done to

see what the amateur players lack and to provide them the knowledge about the same.

The present study thus aimed to compare maximal O₂ uptake (VO₂ max) in amateur and elite badminton players.

MATERIALS AND METHODS

This comparative study was carried out in Navi Mumbai Sports Association and various other sports associations of Navi Mumbai and Mumbai. A total of 50 badminton players (25 elite and 25 amateur) between the age group of 10 to 20 years were included in the study.

Study Design: Comparative/ Analytical study.

Study Location: Navi Mumbai Sports Association, Various other sports associations of Navi Mumbai and Mumba

Study Duration: 6 months.

Sample size: 25 amateur and 25 elite badminton players

Survey method: Convenient sampling

Inclusion Criteria

1. For amateur players: Candidates who have been practicing badminton for less than 1 year.
2. For elite players: Candidates who have been participating at district, state or national level.

Exclusion Criteria

1. Individuals with cardiorespiratory issue
2. Individuals with musculoskeletal injury.
3. Individuals unwilling to participate in the survey

Procedure Methodology

Prior consent was taken of all the subjects of this study and for those who were minors (below the age of 18), an assent form was given to the parent or the guardian. An assessment was conducted among athletes from the age group of 10 to 20. Convenient sampling was done for the same. It was made sure that the athletes are not suffering from any cardiopulmonary disease or recent musculoskeletal injury. The study duration was 6 months.

An ethical clearance was obtained from the institution. The demographic details of each athlete were noted. A brief detail of their level of activity was asked.

The Yo-Yo intermittent recovery test consists of repeated 2 - 20-m runs, back and forth between the starting, turning, and finishing line at a progressively increased speed controlled by audio bleeps from a speaker (BangsboSport.com, Copenhagen, Denmark). Between each running bout, the subjects have a 10-s active rest period, consisting of 2 - 5 m of jogging. When the subjects twice have failed to reach the finishing line in time, the distance covered is recorded and the test results are notes. The test may be performed at two different levels with differing speed profiles (level 1 and 2). In the present study, we have used the Yo-Yo intermittent recovery test, level 1, which consist of 4 running bouts at 10–13 km/h (0–160 m) and another 7 runs at 13.5–14 km/h (160–440 m), thereafter it continues with stepwise 0.5 km/h speed increments after every 8 running bouts (i.e., after 760, 1080, 1400, 1720 m, etc.) until exhaustion.

The test was performed on level ground, marked by cones, having a width of 2 m and a length of 20 m. Another cone placed 5 m behind the finishing line marked the running distance during the active recovery period. All subjects were familiarized to the test by at showing them a video of YYIRT1.

The formula by Bangsbo *et al.*, (2008) for calculating VO₂ max being:

Yo-yo Intermittent Recovery Test 1:

$$VO_2 \text{ max (ml/kg/min)} = IR1 \text{ distance (metres)} \times 0.0084 + 36.4$$

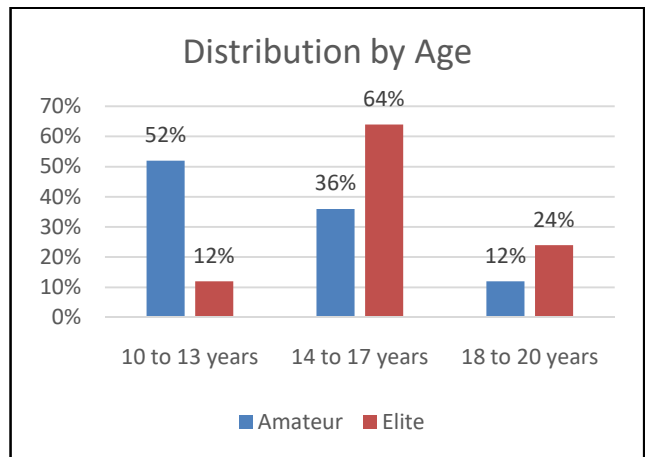
Statistical Analysis

All statistical analysis was done using IBM SPSS version 22.0. A paired sample t test was done on the values of VO₂ max obtained from amateur and elite badminton players. The level *P* < 0.05 was considered as the cutoff value or significance.

RESULTS AND DISCUSSION

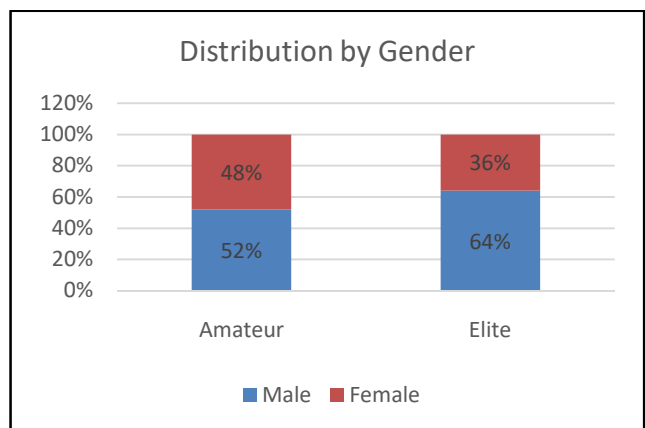
Table 1:

| | Amateur | Elite |
|----------------|---------|-------|
| 10 to 13 years | 52% | 12% |
| 14 to 17 years | 36% | 64% |
| 18 to 20 years | 12% | 24% |



Graph 1:

Inference: As per the graph, 52% of amateur players belong to the category of 10 to 13 years of age whereas 12% elite players belong to the same. Similarly, 36% amateur players are fall into the category of 14 to 17 years of age whereas 64% of elite players fall into the same. Lastly, 12% of amateur players belong to the category of 18 to 20 years of age whereas 24% of elite players belong to the same. (Table 1)

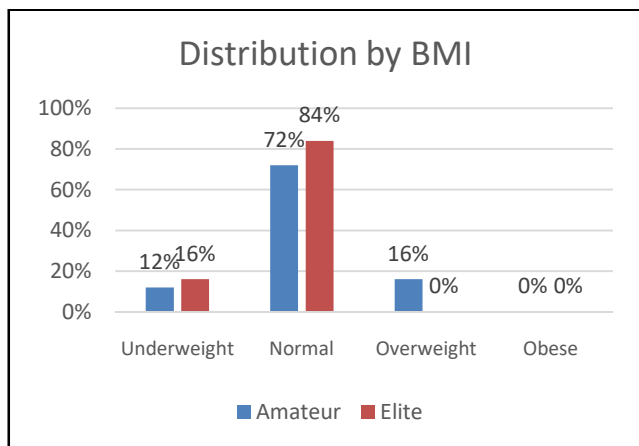


Graph 2:

Table 2:

| | Amateur | Elite |
|--------|---------|-------|
| Male | 52% | 64% |
| Female | 48% | 36% |

Inference: Herein, we can see that there are 48% females and 52% males among amateur athletes and 36% females and 64% males among elite athletes. (Table 2)

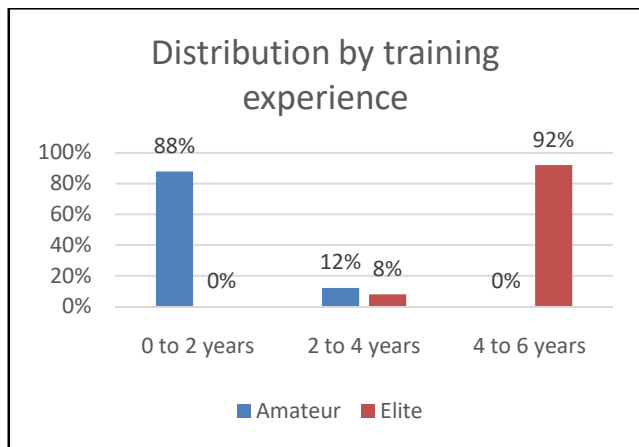


Graph 3:

Table 3:

| | Amateur | Elite |
|-------------|---------|-------|
| Underweight | 12% | 16% |
| Normal | 72% | 84% |
| Overweight | 16% | 0% |
| Obese | 0% | 0% |

Inference: This graph shows that out of the amateur players, 12% are underweight, 72% have normal BMI and 16% are overweight. Similarly, out of the elite players, 16% are underweight, 84% have normal BMI and none are overweight. (Table 3)

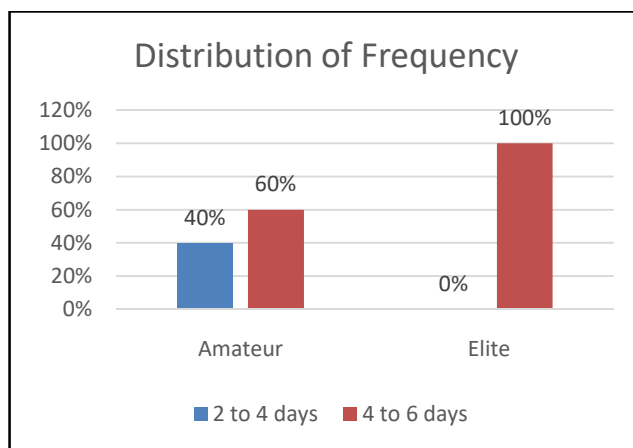


Graph 4:

Table 4:

| | Amateur | Elite |
|--------------|---------|-------|
| 0 to 2 years | 88% | 0% |
| 2 to 4 years | 12% | 8% |
| 4 to 6 years | 0% | 92% |

Inference: This graph shows that 88% of amateur players have been training for 0 to 2 years and 12% of them have trained for 2 to 4 years and none have trained for more than that. Similarly, 8% of elite players have been practicing since 2 to 4 years and 92% of them have been training for 4 to 6 years but none have trained for less than 2 years. (Table 4)

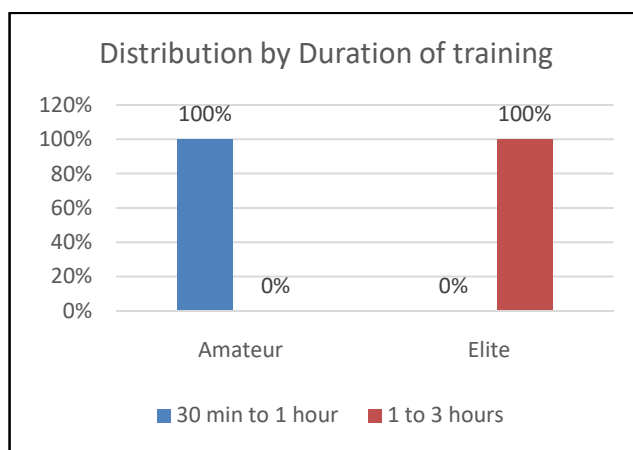


Graph 5:

Table 5:

| | Amateur | Elite |
|-------------|---------|-------|
| 2 to 4 days | 40% | 0% |
| 4 to 6 days | 60% | 100% |

Inference: As per the graph, 40% of amateur players practice for 2 to 4 days and 60% practice for 4 to 6 days. Whereas, all of the elite players practice for 4 to 6 days. (Table 5)

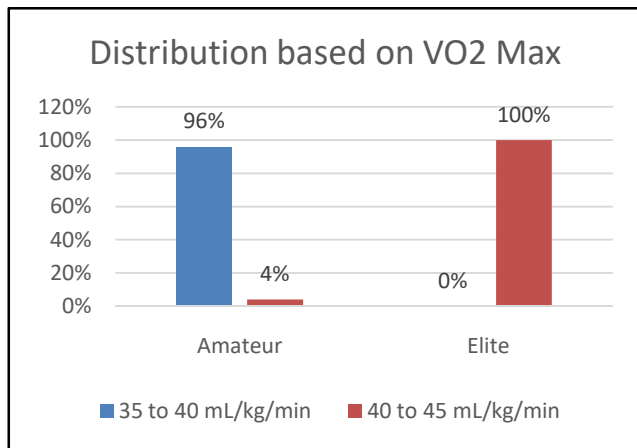


Graph 6:

Table 6:

| | Amateur | Elite |
|------------------|---------|-------|
| 30 min to 1 hour | 100% | 0% |
| 1 to 3 hours | 0% | 100% |

Inference: As per this graph, all the amateur players practice for half an hour upto 1 hour. Whereas, all of the elite players practice for a minimum of 1 hour lasting upto 3 hours. (Table 6)



Graph 7:

Table 7:

| | Amateur | Elite |
|--------------------|---------|-------|
| 35 to 40 mL/kg/min | 96% | 0% |
| 40 to 45 mL/kg/min | 4% | 100% |

Inference: This graph depicts distribution of players depending on VO₂ max. As seen, 96% of amateur players have 35 to 40 ml/kg/min of VO₂ max and 4% of them have 40 to 45 ml/kg/min of VO₂ max. Whereas, all of the elite players have a VO₂ max ranging between 40 to 45 ml/kg/min. (Table 7)

DISCUSSION

Assessment of VO₂ max was performed on 25 amateur badminton players and 25 elite badminton players between 10 to 20 years of age. It was a comparative study wherein the subjects were made to run between 2 cones placed 20 metres apart whilst consecutively walking for a distance of 5 meters with a cone placed 5 meters behind the starting cone.

The subjects were taken from Navi Mumbai Sports Association, Vashi and various other sports associations of Navi Mumbai and Mumbai. All statistical analysis was done using IBM SPSS version 22.0. A paired sample t test was done on the values of VO₂ max obtained from amateur and elite badminton players. A p value of 0.000 was obtained which was considered highly significant.

As per Graph 1, 52% of amateur players between the class interval of 10 to 13 years of age whereas 12% elite players belong to the same. Similarly, 36% amateur players between the class interval of 14 to 17 years of age whereas 64% of elite players fall into the same. Lastly, 12% of amateur players between the class interval of 18 to 20 years of age whereas 24% of elite players belong to the same. This is due to the fact that elite players need to acquire skills that come to them after years of training. Hence, majority of elite players belong to an older age group.

Graph 2 shows that there are 48% females and 52% males among amateur athletes and 36% females and 64% males among elite athletes.

According to Graph 3, out of the amateur players, 12% are underweight, 72% have normal BMI and 16% are overweight. Similarly, out of the elite players, 16% are underweight, 84% have normal BMI and none are overweight. This could be cumulative of a varied number of factors including the type of training they do, the hours they train for, days they practice for or the diet they maintain. According to a study, to gain an advantage in badminton play, the athlete must have a tall, lean and muscular stature with low body fat percentage (Lieshout, 2002). Herein, as we can see, the elite players have a lower BMI giving them an advantage.

According to Graph 4, 88% of amateur players have been training for 0 to 2 years and 12% of them have trained for 2 to 4 years and none have trained for more than that. Similarly, 8% of elite players have been practicing since 2 to 4 years and 92% of them have been training for 4 to 6 years but none have trained for less than 2 years. This is majorly because they require prolonged training experience before they can play the sport at competitive levels.

As per Graph 5, As per the graph, 40% of amateur players practice for 2 to 4 days and 60% practice for 4 to 6 days. Whereas, 100% of the elite players practice for 4 to 6

days. This is possibly due to the fact that the elite players require more regularity and more frequency when it comes to their training sessions since they are playing on a competitive level.

Graph 6 shows that 100% of the amateur players practice for half an hour upto 1 hour. Whereas, 100 % of the elite players practice for a minimum of 1-hour lasting upto 3 hours. Whereas, all of the elite players practice for 4 to 6 days. This is possibly due to the fact that the elite players have a regime of an elaborate warm up before each training session and an extensive cool down session post training. That being there, the training of the elite players is more intensive as compared to the amateur players.

Graph number 7 depicts distribution of players depending on their VO₂ max consumption. As seen, 96% of amateur players have 35 to 40 ml/kg/min of VO₂ max and 4% of them have 40 to 45 ml/kg/min of VO₂ max. Whereas, all of the elite players have a VO₂ max ranging between 40 to 45 ml/kg/min. The evidence available on physiological demands of badminton play suggests that it is a predominantly intermittent aerobic activity with a modest contribution from the anaerobic system; This proves that VO₂ max is an important aspect to be considered during badminton play. (Jorgen *et al.*, 2012)

As we have seen in the earlier graphs, elite players train for a longer duration when compared to amateur players. Also, elite players have more frequent training sessions as compared to amateur players. According to a study done by Astrand and Rodahl in 1986, physical training is the most crucial determining factor in VO₂ max (Glass and Gregory, 2007). Coherently, a study done by Fringer M. N. and Stull G. A. states that endurance training constituting continuous type of exercise can improve the VO₂ max in young adults (Astrand and Rodahl, 1986). Another study states that the players acquire training intensities of 80 to 95% of their maximum aerobic capacity, when training session is for a long duration (30 to 40 minutes) (Fringer and Stull, 1974). Hence, we understand that we need to device a longer duration of training session to enhance the VO₂ max of amateur players.

An article by D Brown, DA Weigland, EM Winter which was done on physiological characteristics of senior and junior squash players states that the VO₂ max of senior players is higher as compared to junior players (Morgan and Pollock, 1977). Hence as in this article we

have obtained a difference in the age range of elite and amateur players, majority of amateur players belonging to a younger age group and majority of elite players belonging to an older age group, we understand that age and level of practise are major determining factors in the consumption of VO₂ max. Hence, from this study, we can establish a better training regime for the amateur players so as to increase their maximal O₂ uptake.

Analogously, a study done by Sergej M. Ostojic states that, elite players had significantly higher estimated VO₂ max values, compared to amateur players and estimated percentage of fast muscle fibers (fast twitch) were higher in elite players as compared to amateur players (Brown *et al.*, 1998). Another study demonstrates that previous glycogen depletion of slow-twitch fibers enhances fast-twitch fiber recruitment, elevates O₂ cost, and causes a slow component of VO₂ during dynamic exercise with no blood lactate accumulation or muscular acidosis. These findings suggest that fast-twitch fiber recruitment elevates energy requirement of dynamic exercise in humans and support an important role of active fast-twitch fibers in producing the slow component of VO₂ (Ostojic, 2004). Hence concluding that VO₂ max consumption is higher in elite players and requires to be enhanced in amateur players for them to be able to continue their play effortlessly for a longer period of time.

CONCLUSION

- VO₂ max in amateur badminton players was 35 to 40 ml/kg/min using yo-yo intermittent recovery test 1.
- VO₂ max in elite badminton players was 40 to 45 ml/kg/min using yo-yo intermittent recovery test 1.
- When VO₂ max of both the groups was compared using paired sample t test, the VO₂ max in elite badminton players was found to be significantly higher (p=0.000) than that in amateur badminton players.

LIMITATIONS

- For this study, we have taken a small sample size due to limitation in the accessibility of elite players.
- Non-uniform distribution of people according to age as majority of amateur players belonged to the age category of 10 to 13 years whereas majority of elite players belonged to the age category of 14 to 17 years.
- Non-uniform distribution of study population as males were more than females in both, elite and amateur categories.

- Improper distribution of people according to BMI as majority of the candidates belonged to normal BMI

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OPACIFIED FOLDABLE INTRAOCULAR LENS AFTER CATARACT SURGERY MANAGED WITH INTRAOCULAR LENS EXCHANGE

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ABSTRACT

A patient presented to us with diminution of vision 2 years after cataract surgery with IOL (intraocular lens) implantation in the right eye. On examination, visual acuity was counting fingers close to face. Slit lamp examination revealed an opacified intraocular lens. IOL exchange was planned with standby anterior vitrectomy and iris claw lens in view of possibility of capsular fibrosis and risk of with-the-bag removal of IOL. IOL exchange was successfully done without damaging the posterior capsule and rigid IOL was placed in the bag. Postoperatively, best corrected visual acuity was 6/9; and the patient was satisfied with the surgical outcome. Opacification of foldable IOL may often be misdiagnosed as posterior capsular opacification. Though rare, IOL opacification has to be considered after careful clinical examination as a cause of poor visual acuity post cataract surgery. It may occur due to calcified deposits on the IOL. Definitive management in the form of IOL exchange can result in good postoperative outcome.

KEYWORDS: Opacified IOL, IOL Exchange

Silicone, hydrogel, and acrylic (hydrophilic and hydrophobic) are the principal materials used for manufacturing IOLs that can be folded to be inserted into the eye through a small self-sealing incision. Dystrophic calcification of IOLs is a rare phenomenon following cataract surgery. Many case reports have been described about such occurrences in literature. (Goodall and Ghosh, 2004). This calcification is seen on anterior surface, posterior surface as well as within the IOL optic. IOL opacification can occur at various periods of time following cataract surgery. Late opacification of foldable IOL may be associated with capsular fibrosis and IOL may have to be removed with the bag. We present a rare case of opacified foldable IOL presenting 3 years after the cataract surgery, for which IOL exchange was successfully done without removal of the bag and rigid IOL was implanted in the bag.

CASE REPORT

A 60 years old female patient presented to us with the complaints of diminution of vision of the right eye since 1 year. She had a history of cataract surgery with intraocular lens implantation in both eyes 3 years ago. The pre-, post-, and intra-operative period was uneventful. The surgeries had good visual outcome. The patient did not undergo any other ocular surgical procedure. The patient was asymptomatic for 2 years postoperatively when she started to notice glare in her vision in the right eye, which gradually progressed to diminished vision in the right eye.

There was no history of any systemic comorbidities. Records of previous cataract surgery were not available.

On examination, best corrected visual acuity of the right eye was counting fingers close to face. Slit lamp examination revealed a clear cornea, quiet anterior chamber with pseudophakia and opacification at the pupillary area which was provisionally diagnosed as posterior capsular opacification. However, careful slit lamp examination after pupillary dilation revealed that the entire IOL itself was opacified. (Figure 1) Fundus could not be seen due to the opacified IOL. Best corrected visual acuity of the left eye was 6/9, slit lamp examination showed a quiet anterior chamber with pseudophakia, fundus examination was within normal limits with a cup disc ratio of 0.4: 1 and a normal foveal reflex.

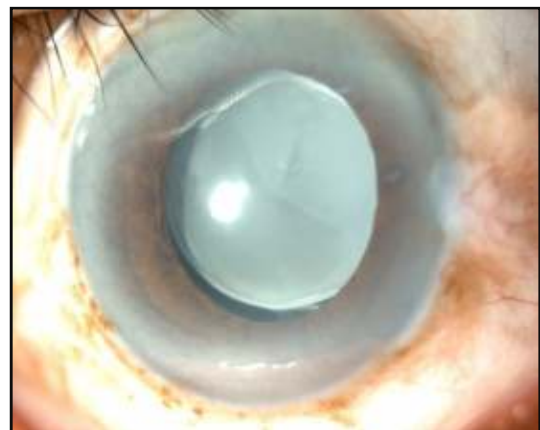


Figure 1: Opacified IOL

¹Corresponding author

IOL exchange was planned for the right eye. Accurate IOL power calculation was done in pseudophakic mode. Since the IOL was implanted 3 years ago, we anticipated difficulty in separating the IOL from the capsular margin. Since there was a possibility of capsular fibrosis precluding the IOL removal, with-the bag IOL removal was considered; anterior vitrectomy and iris claw lens were kept standby.

SURGICAL PROCEDURE

Peribulbar anesthesia was given. A temporal sclerocorneal tunnel was made. Rhexis margin was separated from IOL using iris reposer and ocular viscoelastic. The haptics of the IOL were carefully untucked and brought into the anterior chamber, taking care not to damage the posterior capsule. The opacified foldable IOL was explanted through the scleral tunnel (Figure 2) and rigid IOL was implanted in the bag. (Figure 3) Postoperatively, topical antibiotic and steroid eyedrops (ofloxacin + dexamethasone) were prescribed in tapering dose.

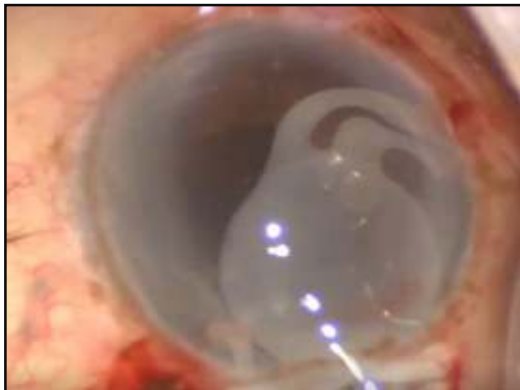


Figure 2: IOL Explantation

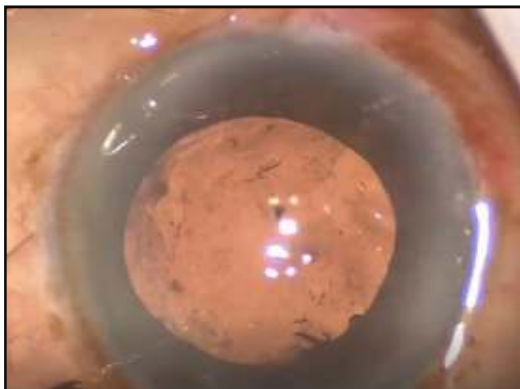


Figure 3: PCIOL in bag

RESULTS

At 2 weeks followup, BCVA was 6/9 and fundus examination revealed an epiretinal membrane. The patient was satisfied with the postoperative outcome.

DISCUSSION

The advent of phacoemulsification and the increasing use of foldable IOLs have given rise to new complications including late opacification of intraocular lenses. This opacification results from calcium phosphate deposits on the surfaces or the internal substance of the lens. Majority of these case reports were seen to occur in patients with asteroid hyalosis and hydrophilic acrylic lenses (Foot *et al.*, 2004). These cases are often misdiagnosed as posterior capsular opacification; with an unsuccessful attempt at YAG capsulotomy. Surgical explantation of IOLs is required in cases of vision threatening opacification. The operative procedure is challenging due to the tight adherence of the IOL to the capsular bag. Complications include zonular dehiscence, rupture of the posterior capsule and corneal decompensation. (Al-Bdour and Dahabreh, 2008) As per our knowledge, this is the first case report in which a 3 year old opacified IOL has been explanted without posterior capsular complications.

Though rare, IOL opacification should be considered as a cause of poor visual acuity post cataract surgery. Careful clinical examination on slit lamp is of paramount importance. Definitive management in the form of IOL exchange has good postoperative visual outcome.

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A CRITICAL STUDY ABOUT THE TOXICITY OF LEAD AND THE PRECAUTIONARY MEASURES: A REVIEW

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ABSTRACT

Lead is the most important toxic heavy element in the environment. Due to its important physico-chemical properties, its use are quite remarkable. Globally it is an abundantly distributed, important yet dangerous environmental chemical. It's important properties like softness, malleability, ductility, poor conductivity and resistance to corrosion seem to make difficult to give up its use. Due to its non-biodegradable nature and continuous use, its concentration accumulates in the environment with increasing hazards.

KEYWORDS: Toxic Heavy Element, Malleability, Ductility, Non-Biodegradable Nature

Human exposure to lead and its compounds occurs mostly in lead related occupations with various sources like leaded gasoline, industrial processes such as smelting of lead and its combustion, pottery, boat building, lead based painting, lead containing pipes, battery recycling, grids, arm industry, pigments, printing of books, etc. Though its widespread use has discontinued in many countries of the world, it is still used in many industries like car repair, battery manufacturing and recycling, refining, smelting, etc. Lead is a highly poisonous metal affecting almost every organ in the body. Of all the organs, the nervous system is the mostly affected target in lead toxicity, both in children and adults. The toxicity in children is however of a greater impact than in adults. This is because their tissues, internal as well as external, are softer than in adults. Long-term exposure of adults can result in activities that measure functions of the nervous system. Infants and young children are especially sensitive to even low levels of lead, which may contribute to behavioral problems, learning deficits and lowered IQ. Long-time exposure to lead has been reported to cause anaemia, and blood pressure, mainly in old and middle aged people. Severe damage to the brain and kidneys, both in adults and children, were found to be linked to exposure to heavy lead levels resulting in death.

WHAT IS TOXICITY

Toxicity is the degree to which a chemical substance or a particular mixture of substances can damage an organism. Toxicity can refer to the effect on a whole organism, such as an animal, bacterium, or plant, as well as

the effect on a substructure of the organism, such as a cell (cytotoxicity) or an organ such as the liver (hepatotoxicity).

Sometimes the word is more or less synonymous with poisoning in everyday usage. A central concept of toxicology is that the effects of a toxin are dose-dependent; even water can lead to water intoxication when taken in too high a dose, whereas for even a very toxic substance such as snake venom there is a dose below which there is no detectable toxic effect. Toxicity is species-specific, making cross-species analysis problematic. Newer paradigms and metrics are evolving to bypass animal testing, while maintaining the concept of toxicity endpoints. Toxic effects holder importance when plants, animals and human beings are considered, along with their surrounding environment.

TYPES OF TOXICITY

There are generally four types of toxic entities, *Vis.*; chemical, biological, physical and radiation:

- Chemical Toxicants include inorganic substances such as, lead, mercury, hydrofluoric acid, and chlorine gas, and organic compounds such as methyl alcohol, most medications, and poisons from living things. While some weakly radio active substances, such as uranium, are also chemical toxicants, more strongly radioactive materials like radium are not, their harmful effects (radiation poisoning) being caused by the ionizing radiation produced by the substance rather than chemical interactions with the substance itself.
- Disease-causing microorganisms and parasites are toxic in a broad sense, but are generally called

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pathogens rather than toxicants. The Biological Toxicity of pathogens can be difficult to measure because the "threshold dose" may be a single organism. Theoretically one virus, bacterium or worm can reproduce to cause a serious infection. However, in a host with an intact immune system the inherent toxicity: of the organism is balanced by the host's ability to fight back. Nonliving biological toxicants are generally called toxins if produced by a microorganism, plant, or fungus, and venoms if produced by an animal.

- Physical Toxicants are substances that, due to their physical nature, interfere with biological processes. Examples include coal dust, asbestos fibers or finely divided silicon dioxide, all of which can ultimately be fatal if inhaled. Corrosive chemicals possess physical toxicity because they destroy tissues, but they're not directly poisonous unless they interfere directly with biological activity. Water can act as a physical toxicant if taken in extremely high doses because the concentration of vital ions decreases dramatically if there's too much water in the body. Asphyxiate gases can be considered physical toxicants because they act by displacing oxygen in the environment but they are inert, not chemically toxic gases.

TOXICITY OF ELEMENT LEAD

Several methods are used to detect elevated blood lead levels. The presence of changes in blood cells visible under the microscope or deletion of dense lines in the bones of children seen on X-ray are signs used for detecting lead poisoning. However the main tool to detect elevated levels of body lead is to measure the level of lead in blood samples. This test gives however only an account of lead present in circulating blood but cannot show how much lead is stored in the body. As of 2012, the Centers for Disease Control and Prevention (USA) have set the standard elevated blood lead level for adults to be 10 µg/dL and for children 5 µg/dL of the whole *blood*. Previously, the standard lead level for children was 10 µg/dL. The appearance of clinical manifestations varies from individual to individual depending on other environmental factors. In some there is a clear appearance of clinical features even at lower levels, while some are asymptomatic even at higher levels of lead present in their body fluids. Children are more prone to the effects of lead because usually their

organs are in a developing stage. Thus blood lead levels have to be set lower and must be frequently checked, particularly where contamination is expected.

HARMFUL EFFECTS ON FOOD, HEALTH AND ENVIRONMENT

All along human history, lead poisoning has been reported to have severe effects. Occasional lead poisoning was found to be caused by lead salts used in pottery glazes leached by acidic fruit juices. It is also assumed that in the eighteenth and early nineteenth century lead was illegally added to wine both as a sweetener and to make it appear fresh. Lead poisoning is believed to be primarily responsible for the collapse of the Roman Empire, in which lead acetate was used as a sweetener of wine. Its prolonged use was considered to have caused dementia to many Roman emperors. Lead poisoning has, also been found to be the cause of anaemia in a number of cases as lead inhibits porphobilinogen synthase and ferrochelatase, preventing both porphobilinogen formation and the incorporation of iron into protoporphyrin IX, which prevents heme synthesis. One of the mechanisms by which lead interferes with cognition is that it acts as calcium analogue which interferes with ion channels. It has been observed that Pb²⁺ is a potent reversible and selective: blocker of voltage-dependent calcium channels at low concentrations. It has been known that the toxic effects on blood cells of rates caused by lead nitrate was alleviated by sodium selenite. They also showed that effects of lead nitrate were more harmful in diabetic than in non-diabetic rats. Oxidative stress was studied by low level lead exposure in first grade Uruguayan children, suggesting its lead has potentially adverse effects on oxidative stress.

Impaired respiratory function was observed in workers exposed to lead with elevated blood lead concentration and zinc protoporphyrin concentration.

PRECAUTIONARY MEASURES

Lead poisoning causes severe effects and is a matter of serious concern, yet importantly, it is preventable. The best approach is to avoid exposure to lead it is recommended to frequently wash the children's hands and also recommended to discourage children from putting their hands, which can be contaminated, in their mouth habitually, thus increasing the chances of getting poisoned by lead. Vacuuming frequently and eliminating the use and

or presence of lead containing objects like blinds and jewellery in the house can also help to prevent exposures. House pipes containing lead or plumbing solder fitted in old houses should be replaced to avoid lead contamination through drinking water. It is believed that hot water contains higher lead levels than does cold water, so it is recommended that for household uses cold water should be preferred to hot water. Dimercaprol and succimer constitute the treatment process for lead poisoning.

Lead poisoning is generally treated by using chelating salt disodium calcium edentate, which is the calcium chelate of the disodium salt of ethylene-diamine-tetracetic acid (EDTA). Such chelating agents have a great affinity to the removing agent. The chelating agent for lead has a greater affinity to lead than calcium and so the lead chelate is formed by exchange. This is then excreted in urine, leaving behind harmless calcium. Blood lead levels were shown to be lowered by treatment with succimer used as chelation therapy in children exposed to lead to improve their neuropsychological development. And yet, though succimer was observed to help in reducing blood lead levels, it failed in improving the scores of cognition tests (Rogan *et al.*, 2001). There is a number of antioxidants which are believed to act against toxicity of chemicals like lead and its related compounds. A new technique called nano-encapsulation of antioxidants may provide improved biodistribution and bioavailability of poorly soluble therapeutics through solubilisation (Flora *et al.*, 2012).

Encapsulation of curcumin in a pluronic block copolymer demonstrated a slow and sustained release of curcumin and showed anticancer activity comparable with free curcumin (Sahu *et al.*, 2010). These new techniques may hold a promise for treating a number of human diseases. In a very recent study, it was observed that puerarin promoted Akt and GSK-3J3 phosphorylation in PC12 cells exposed to lead acetate. The authors of the study concluded that puerarin as a phytoestrogen might be an attractive agent for prevention and treatment of chronic diseases related to lead neurotoxicity. In another recent finding beta-carotene was observed to have an antioxidant action and exert some beneficial effects in lead poisoning, independent of chelation (Dobrkowski *et al.*, 2014). The authors also found significantly decreased homocysteine levels due to administration of betacarotene in lead exposed workers. Recently a study on a group of workers occupationally exposed to lead found that those treated with

N-acetylcysteine (NAC) showed a significant reduction in their blood lead levels. In addition, all groups receiving NAC were shown to have significantly elevated activity of glutamate dehydrogenase. It was further reported that treatment with NAC normalised the level of homocysteine and decreased oxidative stress. It was thus concluded that NAC could be recommended as an alternative therapy for chronic lead toxicity in humans.

DISCUSSION AND CONCLUSION

Of all the heavy metal poisonings, lead poisoning appears to be rather prominent. The use of lead has been evidenced from ancient times and its toxicity reports are well documented. Due to its important physico-chemical properties, it has been used all over the world. With the onset on industrialization from the seventeenth century onwards, its use increased manifold, leading to increased toxicity in humans. Children are at a higher risk, particularly at sites where lead related occupations are nearby their playing grounds. Workers who are occupationally exposed to lead are also at increased risk of lead poisoning. Children of parents who are occupationally exposed to lead should be frequently checked for lead levels in their blood to avoid lead related risks. Lead toxicity is evident from the literature and there is almost no function in the body which is not affected by lead. Lead toxicity disrupts the functions of the digestive system, nervous system, respiratory system, reproductive system, *etc.* In addition, lead prevents enzymes from performing their normal activities. Lead even disrupts the normal DNA transcription process and causes disability in bones. Lead as such has no physiological role in the body and even smaller levels of lead can cause toxicity. The good news is however that it can be reversed and the levels of lead can be reduced from the body by a number of techniques used nowadays. The prominent ones among them are chelation therapy, nano-encapsulation, N-acetylcysteine (NAC). A number of antioxidants also help in the removal of lead from the body. Though there are several methods of treatment available nowadays, it is certainly better to prevent direct exposure to toxins and thus preclude future consequences. It is also recommended that parents should educate their children about how to prevent accidental lead poisoning. The treatment strategies are not equally effective for everybody due to the differences ranging from genetic factors to environment and diet.

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ROLE OF PHYLLOSPHERIC BACTERIA IN BIOCHEMICAL MECHANISM OF SALINITY STRESS ALLEVIATION IN *Centella asiatica*

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ABSTRACTS

Phytochemical or biochemical preparation of various plants like *Centella asiatica* contains various biologically active compounds and its analysis will provide an opportunity for new drugs lead. Due to their medical importance, there is always a need to cultivate these plants at large scale. In current scenario high population explosion demands for more crop productivity, but crop productivity is adversely affected by increasing abiotic stress like soil salinization. High salinity adversely affects plant growth because it increases osmotic potential gradient of soil which further decreases water and nutrient uptake ability of plant roots from soil. Low water and nutrient availability inhibits various vital activities of plant. Various important amino acids (like proline, methionine etc) help plant to alleviate or tolerate abiotic stresses like soil salinity. Abiotic stress tolerance mechanism in Plant cells include remodeling of their gene expression which results in corresponding alteration in the intracellular levels of such stress alleviating metabolites like amino acids in plant cells. Development of methods, to improve stress tolerance against soil salinization in plants, is a strategy to deal with negative impacts of harsh environmental conditions on plants. Exploitation of beneficial microflora present in phyllosphere of plant provides a new platform for improving salinity tolerance in the plant. Bacteria are the most abundant inhabitants of the phyllosphere. This review focuses on stress alleviation mechanism attributed by Phyllosphere bacteria in plant. Recent biochemical and molecular studies in plant biotechnology have provided insights into metabolic network of plant-microbe interaction that enhance salinity tolerance. The significance of phyllosphere microbiome includes regulation of biosynthesis of stress alleviating sulfur containing metabolites like amino acids, vitamins in host plant. Sulfur metabolites are useful to alleviate salinity stress in plants due to their ion scavenging ability which results in osmotic balance. This review signifies the role of phyllospheric bacteria in remodeling of various metabolic activities of host plant that confer salinity tolerance

KEYWORDS: Abiotic Stress, Abiotic Stress Tolerance, Amino Acids, *Centella asiatica*, Phyllosphere, Soil Salinization, Sulfur Containing Metabolites

In present Anthropocene era, climatic conditions are continuously changing due to various human activities which further created many environmental stressors like soil salinity. Water and land are two vital parameters for agriculture. Salinity involves increase in salt concentration of soil and water and this excessive salt concentration negatively affects crop productivity worldwide. So, Agricultural organizations conduct many researches to develop a significant control mechanism which helps to influence stress tolerance activity of plants against salinity. By increasing the productivity of commercially significant crop plants we can fulfill the demand of large scale world population. One of the economically and commercially important plants is *Centella asiatica*. There were varieties of researches conducted previously on *Centella asiatica* plant. Among these, certain reviews are concerned with detail study of morphology, geographical distribution, phytochemical characteristics and composition of *Centella asiatica* plant (Singh *et al.*, 2010). It is used as a culinary vegetable and medicinal herb. The plant has great economical and commercial significance. Large scale

cultivation of this plant is required by commercial cultivars. In current scenario soil salinization has affected a large proportion of agricultural land and become an important obstacle to achieve the desired yield of such commercial plants. Significant reviews and informations are available which focus on certain factors which facilitate or promote plant growth by increasing stress tolerant mechanism against salinity. Among these factors, plant microbiome plays an important role in plant growth promotion. Phyllospheric or arial parts of plant body extensively populated by varieties of microflora. Some phyllospheric microflora especially bacteria exerts positive impacts on host plant metabolism and growth. The present review involves study and evaluation of growth promoting phyllospheric bacterial population of *Centella asiatica* (Muller *et al.*, 2003) and also elaborates their role in enhancement of stress tolerance mechanism of the plant against salinization. Such studies lead the development of various biological preparations containing phyllospheric bacterial inoculants which promote stress tolerance

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mechanism in host plants and further improve crop productivity.

Centella asiatica

Centella asiatica commonly called as Centella, Gotu kola or Asiatic pennywort. It is a herbaceous, perennial plant of family Apiaceae. It is native to wetlands in Asia. It grows in temperate and tropical swampy areas in many world regions. Many reviews help us to know economical and medical significance of *Centella asiatica*. *Centella asiatica* plant extract contains various biologically and pharmacologically active compounds, which are known to have therapeutic effects against various diseases (Brinkhaus *et al.*, 2000). It also has potential to monitor various heavy metal pollution levels in soil and to control various species of weeds. The plant is a small trailing herb and it is the only species of *Centella* found in India. Its stem is glabrous, pink striated and rooting at nodes. Leaves of the plant are fleshy, orbicular to reniform and dentate. Petiole is long, smooth on upper surface and hairy below. Flowers are pink and white in fascicled umbels. The fruits are oblong, dull brown and laterally compressed pericarp hard, thickened and woody. *Centella* contains pentacyclic triterpenoids, asiaticoside, brahmoside, asiatic acid and brahmie acid (Hashim *et al.*, 2011). These phytochemicals are used to treat various disorders, minor wounds, to encourage lactation, headache, nausea, and drowsiness etc (James *et al.*, 2009). Previous researches also revealed beneficial role of *Centella asiatica* in epithelial cell proliferation and help in sound healing (Ruszymah *et al.*, 2012). *Centella asiatica* plant is already studied for having anxiolytic properties (Wijeweera *et al.*, 2006).

ABIOTIC STRESSES AND THEIR IMPACT ON PLANT

(In special reference of Soil Salinization)

Like all forms of life, plant growth is also depends on its surrounding. Plant surrounding consists various biotic and abiotic factors which altogether affect plant growth. The non living variables of any environment are known as abiotic factors. Abiotic factors are natural, intangible and inanimate factors like temperature, drought, sunlight, wind and soil salinity. When the level of these abiotic factors varies from their optimum range, it creates an abiotic stress on plant growth and productivity (Negrao *et al.*, 2017).

Among these soil salinity or soil salinization is a major abiotic stress which significantly affects plant growth (Hirayama *et al.*, 2010). Soil salinization is an accumulation of water soluble salts to extents that adversely affect plant growth and productivity. Soil composition is very important for plant growth because it consists water and various minerals which promote plant growth. Plant uptakes water and minerals from soil by its roots. High salinity is harmful for plant because it alters or increases osmotic potential gradient which leads excessive transportation of various ions (Na^+ , Cl^-) from soil to plant cells. High salinity of soil also causes over production of reactive oxygen species (ROS) that leads increased redox state of plant cell. Consequence of intracellular accumulation of ROS is breakdown of various biomolecules like protein, lipid, nucleic acid etc in plant cell which results cellular death and diminishes productivity of plants. Reviews show that the higher concentration of salts in soil decrease germination ability of *Centella asiatica* plant seeds (Devkota *et al.*, 2010).

ABIOTIC STRESS TOLERANCE IN PLANT

Plants has inbuilt mechanism to alleviate the harmful impacts of various abiotic stresses like soil salinization. This stress alleviation mechanism of plants against soil salinity involves role of various plant metabolites (Gill *et al.*, 2009). Plants are able to sense any change in their environment and respond accordingly. This response involves complex network of cell signaling to regulate gene expression which further leads appropriate modification in metabolic pathways in plant cells (Kumar *et al.*, 2017). Modified concentration of various metabolites help to leach out toxic ions from plant cells and protect them from adverse impact of soil salinization. Significant informations are available which revealed the contribution of various amino acids like proline (Szabados *et al.*, 2009), methionine and its related compounds (Ogawa *et al.*, 2011) in alleviation of soil salinity stress by various plants.

PHYLLOSPHERE MICROFLORA

Plant body provides a suitable habitat for microbial growth and development. Like roots, above ground structures of plant body are also highly colonized with varieties of micro organisms (Andrews *et al.*, 2000). Microbial inhabitants that grow on aerial parts of plant body called phyllosphere. This phyllospheric microbial

community is diverse and affected by host plant itself (Vorholt *et al.*, 2012). Knowledge based on previous researches shows that leaves of plant are highly populated by varieties of microorganisms like bacteria, fungus, algae etc. Leaf of a plant exposed to fluctuating physical parameters like temperature, humidity etc. varieties of bacteria are able to resist this hostile environment on of leaf and populate it. Some bacteria grow on surface of leaves called epiphytic bacteria (Fürnkranz *et al.*, 2008) while others are endophytic bacteria that invade interior of leaf. Many culture methods used to isolate microflora from plant phyllosphere (Mazinani *et al.*, 2017). Cultured microflora has been identified by using biochemical tests. Due to continuous advancements in biotechnology microbial population can be identified at genetic level by using high throughput sequencing methods (Xie *et al.*, 2015). Various studies suggested that phyllospheric micro flora has direct interaction with host plant (Kembel *et al.*, 2014). Sometimes plant and its phyllospheric microbes interact symbiotically and benefit each other. Some species of phyllospheric bacteria have potential to facilitate host plant growth, development and fitness.

SALINITY TOLERANCE MEDIATED BY PHYLLOSPHERIC BACTERIA

As we know that plant microbiome helps to improve physiological and biochemical activities in host plant (Numan *et al.*, 2018). Microbial population populates plant body, grow on host plant and perform various metabolic procedures. During microbial metabolism various kinds of products and metabolites are formed. Microbial metabolites produced by plant microbiome play role in plant microbial interactions. Phyllospheric microflora makes an essential part of plant microbiome and always provides a base for studies which help to find out advance methods of crop improvements. Researches showed that phyllospheric bacterial populations are known to have significant role in stress tolerance mechanism against soil salinity by host plants (Jalili *et al.*, 2009). Plant cells synthesize elevated concentration of certain metabolites under abiotic stress conditions which help them to adopt such stress conditions. Various researches specified effects of phyllospheric microbiome on host plant metabolism and regulate biosynthesis of various abiotic stresses alleviating metabolites which contribute in abiotic stress alleviation (Ruppel *et al.*, 2012).

PLANT METABOLITES HELP IN STRESS TOLERANCE

Salinity is an important abiotic stress which negatively affects growth at physiological, metabolic and molecular levels and decrease crop productivity. Plants are adopted various mechanisms to alleviate the negative impacts of high salinity on their productivity. Plants cope with high redox and osmotic stress caused by salinity through improved metabolism which further maintains higher levels of certain metabolites in cell. Living organisms consist various essential elements like C, H, O, N, S etc. Among all these elements S occupies fourth place. Sulfur is an important element of various plant metabolites like amino acids, vitamins, polysaccharides, cofactors etc. These sulfur containing metabolites regulate wide range of vital processes in plants. Researches suggested that intracellular levels of sulfur containing metabolites in plant cells play major role in stress tolerance mechanism against salinization due to their ion scavenging properties (Khan *et al.*). Sulfur containing amino acids like methionine, cysteine is known to as an essential precursor of metabolic pathways of sulfur metabolism. So, such amino acids have significant importance in signaling and regulation of sulfur metabolism under salinity stress conditions. Studies revealed salinity stress tolerance of plant has been correlated with increased concentration of sulfur containing amino acids. In current scenario, it is necessary to focus our studies on certain factors that induce sulfur metabolism and develop new methods to salinity tolerance in plants. Phyllospheric bacterial population of plant is known to induce stress tolerance ability (Lata *et al.*, 2018).

CONCLUSION

Production of desired quantity of commercially significant crops for global population requires sustainable agricultural practices which benefit producers, consumers and protect environment also. But, salinity is a major constraint to achieve this objective. In Anthropocene era, salinity is prevailing in environment due to many reasons. Crop plants are able to adopt themselves and tolerate high salt concentration of soil that results in increased productivity of the crop. It is necessary to develop control measures which lead higher productivity of various commercial cultivars like *Centella asiatica*. Plant associated microbiome especially phyllospheric bacteria can significantly contribute in metabolic and biochemical

mechanism against salinity stress and conferring elevated tolerance to host plant. Such plant friendly bacterial inoculants can be used as a biocontrol agent against salt stress. Despite informations and reviews are available in this field, more studies and researches are required in future. So, potential biocontrol agents can be developed which facilitate sustainable agricultural and organic farming practices.

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THE PSYCHOACOUSTIC PROPERTIES OF SOUND: AN INTRODUCTION

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ABSTRACT

This study presents a general review on four psychoacoustic properties of sound: pitch, loudness, duration and timbre. Most studies in this domain provide an extensive detail on the topic; however, this article presents a perspective to the beginner on how sounds and/or music can be influenced by these properties. This article has made an effort to provide a preliminary understanding of the domain by highlighting concepts associated closely with cognition of music. The present study is largely confined to four major psychoacoustic properties of sound and their effect on music perception and cognition. A review of existing literature was performed with the purpose of providing insight into the topic under study.

KEYWORDS: Psychoacoustics, Music Cognition, Psychological Acoustics

This article explores the area of psychological acoustics, also known as psychoacoustics. The scholarly materials selected for review in this paper are examined on the basis of four major psychoacoustic properties of sound, that is Pitch, Loudness, Duration and Timbre. Each of these sonic properties offers important insights on the perception and cognition of music and sounds.

PSYCHOACOUSTICS

This review article aims to examine the area of psychoacoustics. Rossing (2007) defines psychoacoustics as being the area of study which is involved in exploring the relationship between physical properties of sound and their perceptual attributes. It is that area of auditory research where behavioral methods are used to express how well listeners perceive sound (Buell, Trahiotis and Bernstein; 2009). Research on the effects of music on humans is made easier due to the fact that humans are naturally musical, an argument Schulkin and Raglan (2014) suggest. Schulkin and Raglan (2014) argue that music plays a pivotal part in our evolution and that we sang before we spoke in grammatically arranged sentences. Similarly, Sloboda (2005) assumes since music and sound are a good example of something else i.e. “a complex motor skill; a language-like phenomenon; a complex auditory phenomenon; a set-theoretic entity” (p.101) researchers are interested in its attributes. Through their study, Levitin and his colleague demonstrated that music has physiological benefit too. Levitin and Menon (2005) were among the first people to explain the role of nucleus accumbens and ventral tegmental area along with hypothalamus and insula in music listening. Nucleus accumbens is the part of human brain that is involved in forming reward related behaviors

(Day and Carelli, 2013). In one of his groundbreaking research, he and his colleague have also demonstrated, for the first time, that musical structures are processed in the language area of the brain (Levitin and Menon, 2003). Emphasizing on the deep-rooted nature of music on human brain, Oliver Sacks, a renowned British neurologist, affirms that even if someone suffers severe brain damage or injury, music is the last thing they lose. Music shapes our brain so much so it involves numerous parts of the brain including those of emotional, motor and cognitive areas (Sacks, 2006).

MAJOR PROPERTIES OF SOUND

Physical dimensions of sound give rise to its psychological features. Elaborating on the perceived parameters of sound, Moylan (2014) writes, “Our perception of sound is a result of the physical dimension being transformed by the ear and interpreted by the mind. The perceived parameters of sound are our perceptions of the physical dimension of sound” (p. 16). In particular, the perceived parameters of Frequency, Amplitude, Time and Timbre are Pitch, Loudness, Duration and Timbre (perceived overall quality), respectively. The following section will analyze the role of the four properties of sound – pitch, loudness, duration and timbre– and their influence on music perception and cognition.

Pitch

One of the most crucial aspects of sound is Pitch. Music without pitch would be drumbeats, speech without pitch processing would be whispers and identifying sound sources without using pitch would be severely limited (Yost, 2009). Pitch is one of the main dimensions along

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which a sound varies in a musical piece. Other dimensions are important as well, although the link between basic science and music is strongest in the area of pitch, mainly because much is known about how pitch is analyzed by the auditory system (McDermott and Oxenham, 2008).

Any sound or melody is recognized even when the notes are shifted upwards or downwards in pitch by the same amount (McDermott and Oxenham, 2008). Thompson (2013) writes, "Shifting the pitch of a single note of a melody is highly noticeable, even when it only alters the original pitch by one semitone" (p. 120). Tan *et al.*, (2010) distinguish pitch in terms of two-dimensions: pitch height and chroma. Pitch height is the frequency of vibrations, whereas chroma refers to a category represented by a certain pitch: "The name we give to the notes in western tonal music (e.g. C, D, E) refer to the pitch chromas" (p. 74). The tones that are separated by an octave exhibit the same chroma, whereas within the same octave, change is referred to in terms of pitch height (Tan *et al.*, 2010). Also, Seashore points out various factors – such as physiological limit, relation to intelligence, relation to age, relation to training, inheritance, frequency level and sensation level, binaural versus monoaural discrimination, duration and masking – that define pitch discrimination, also known as a sense of pitch (Seashore, 1967). Our ability to discriminate between two different pitches can be explained in terms of *place* theory of pitch perception. The perceived pitch of a sound can be directly understood in terms of the place of maximum excitation caused in the basilar membrane. Occasionally, two tones may have similar frequency so much so that it may overlap the same area in the basilar membrane, termed as the critical band. However, the smallest change in frequency that a listener can detect, known as just noticeable difference, may as well determine pitch perception. As the gap between frequencies exceeds the critical band, tones are perceived as being different. An alternative to place theory, the periodicity theory of pitch perception, suggests that the time interval in which the signal repeats, determines its frequency. As a matter of fact, the controversy persists between place and periodicity theorists (Sethares, 2005). All things considered, pitch and frequency should not be considered to be the same. The mathematical concept of frequency and pitch as musical tones are used interchangeably (Walker and Don, 2013), however, "The pitch of a sound corresponds to the

frequency of the sine tone that is judged to have the same pitch" (Beauchamp, 2007) (p. 33).

Loudness (Intensity)

Loudness is understood in relation with its physical variable – amplitude, although other variables may also have an effect. Amplitude is commonly measured in decibels (dB) and is expressed as sound pressure level, SPL (Hodges and Sebald, 2011). Unlike most other scales, the decibel scale is logarithmic. In a decibel scale if we go up three decibels, then we double the volume of the sound. Which is to say, a 103dB sound is twice as loud as a 100dB sound. The three decibels increase in sound pressure, doubles the power of sound however, ten decibels increase in sound, multiplies the sound power ten times, but human ear perceives it only twice the increase in original loudness (Utz, 2003). With every 10dB increase, the power of a sound increases by the factor of 10 (Smith, 2003).

Loudness perception is not a unitary phenomenon but is influenced by the nature and the context of the sound in terms of its psychoacoustic effect (Howard and Angus, 2006). Above all, our judgment of loudness is comparative besides being subjective in nature. The objective measure of sound pressure level is certainly not equivalent to subjective measure of loudness sensation (Beament, 2001) (Howard and Angus, 2006) (Meyer, 2009) (Florentine, 2011). Meyer (2009) suggests, "When tones of different frequency are compared to a tone of 1,000 Hz, the so called equal loudness curves are obtained" (p. 7). These curves explain the relationship between objective sound pressure level and the loudness level as perceived by the ear. Commonly, loudness is measured in sone. "One sone is defined as the loudness of 1-kHz tone at 40-dB SPL heard binaurally in a free field from a source in the listener's frontal plane" (Florentine, 2011) (p. 4). Florentine (2011) further adds that tones with loudness of 2.0 sonas is twice as loud as 1-kHz, 40-dB SPL and the tones having loudness of 0.5 sonas is half as loud.

In early 1930's, Fletcher and Munson at Bell Laboratory conducted a study to examine, "How louder or softer different frequencies had to be in order to be perceived as loud as 1 kHz" (Izhaki, 2012) (p.11). Fletcher-Munson curves, generally known as equal-loudness contour are the result of loudness matching, one of the two methods used to measure loudness perception. In this technique, the subject may be asked to compare the

intensity of a presented tone to a standard tone. The study conducted by Fletcher and Munson had 1 kHz as standard tone. If the similar process is repeated for number of other frequencies the outcome will result in equal-loudness contour (Mather, 2006). Loudness of different frequencies is represented through equal-loudness contour, however through loudness scaling method, the measurement of loudness with its increase in intensity is defined. In this method, the subject is asked to assign numbers to sounds with respect to the standard tone. That is, whether the presented tone is 100 times or 200 times as loud as the standard tone and so on, the technique known as magnitude estimation (Moore, 2013). Equal-loudness contour shows that highs and lows of any sound are more prominent with high volumes. On the contrary, at low volumes the mid range frequencies sound more prominent. Generally, low volumes are associated with power whereas highs with definition, clarity and spark (Izhaki, 2012). This can be understood in terms of the 'bass boost' function in audio equipment. The 'bass boost' function in an audio equipment accentuates low frequencies at low intensities making it relatively louder at the same intensity (Mather, 2006).

Duration (Time)

Time is one of the important aspects as it gives life and structure to music (Tan *et al.*, 2010). In order to understand duration and timing in music, component concepts such as beat, tempo, meter and rhythm need to be defined (Hodges and Sebald, 2011).

Beats are psychological organizing feature of music. Steady beats provide a framework for the listener to organize the music whereas unsteady beats might prove to be psychologically distressing. Research shows that the ability to extract a regular pulse from a piece of music is universal in humans (Hodges and Sebald, 2011). Aniruddh Patel, Professor of Psychology at Tufts University, states that "synchronization to a musical beat relies on the brain systems designed for vocal learning involving auditory-motor networks not restricted to the cortex" (as cited in Altenmuller *et al.*, 2013) (p. 138). Second most important aspect in duration is tempo. Tempo is the rate at which the beat occurs. Commonly, a tempo under 60 beats per second is considered slow. Furthermore, tempo slower than 42 beats per minute and faster than 168 beats per minute is rarely used in music (Hodges and Sebald, 2011). Tempo has its role in evoking emotions in its listeners. Even

though the perception of emotions through tempo varies across cultures, generally slower tempo are associated with sadness, melancholy, low spirit and other unpleasant emotions whereas fast tempo is associated with pleasant emotions including happiness, excitement and so forth (Levitin *et al.*, 2018). Similar concept is that of Meter. Meter refers to the arrangement of strong and weak beats in a musical piece. Broadly, the listeners of western music divide the meter into two's and three's even though there are no strong or weak beats in it. This phenomenon is defined as subjective rhythmization. 100 ms (millisecond) or one tenth of a second is the shortest interval in which we can organize beat metrically, such that if the beats are 1800 ms (nearly two seconds) apart, the tempo is too slow to organize (Hodges and Sebald, 2011). Finally, Rhythm is defined as time patterns created by notes as music unfolds over time (Tan *et al.*, 2010). However, Tan *et al.*, (2010) argue that time durations are not the primary generators of rhythms. Hodges and Sebald (2011) affirm, rhythm in music is so important that sometimes we just identify a melody by just its rhythmic pattern. The important fundamental characteristic of rhythm is that they are based on relative time than absolute time, absolute time meaning time-span with no comparison like that of stopwatch. Rhythm remains constant even when the tempo in a musical piece changes. Therefore, rhythm cannot depend on absolute time as absolute time changes with the change in tempo (Tan *et al.*, 2010). These components— beat, tempo, meter and rhythm – make up the duration in a musical piece.

Timbre (Tone Color)

Timbre is the perceived sound quality through which the listener can distinguish between sounds having similar pitch and loudness as being dissimilar. It is a unique attribute of sound that allow humans and other animals to distinguish among different sound sources on the basis of their perceptual qualities such as pitch, loudness and duration (Patil *et al.*, 2012). Timbral descriptors of sound include, "mellow, rich, covered, open, dull, bright, dark, strident, grating, harsh, shrill, sonorous, sombre, colorless and lacklustre" (Howard and Angus, 2006) (p. 216).

A major component of timbre is the dynamic envelope. It can be understood as the contour of the changes in the overall dynamic level of the sound with: Attack, Decay, Sustain and Release, as its components.

These components may or may not be present in any sound source. In fact, some musical instruments may have more parts of these in their envelope than other instruments (Moylan, 2015). Timbre is mostly dependent on signal shape, although it can be affected by numerous other physical variables (Hodges and Sebald, 2011). It has two broad characteristics that contribute to music perception. Firstly, it is the multitudinous set of perceptual attributes that are changing throughout the musical piece (e.g. attack, nasality, brightness etc.) also others which are discrete or categorical (e.g. 'blatt' at the beginning of a trombone, pinched offset of harpsichord etc.). Secondly, it is a vehicle to track, recognize and identify a sound source over time which gives the listener absolute categorization of the sounding object (McAdams, 2013). Timbre is related with the harmonic structure of a sound or a tone. It hugely depends upon tone's attack and decay pattern. However, to distinguish the tone between the same instruments having similar attack and decay pattern might be difficult. While identifying the sounds of musical instruments, if the onset and offset phases of notes are removed, then listeners may find it problematic to distinguish between sounds. In fact, the onset phase of stringed instrument will be different to brass instrument; the percussion will differ from wind instruments. The onset and offset phases provide an acoustic cue to identify the timbral quality of an instrument (Howard and Angus, 2006). For instance, if an audio recording is played backwards, even though its spectrum remains the same, it will sound completely different. This demonstrates the importance of time envelope in determining the timbral feature (Sethares, 2005).

Timbre can be defined as that quality of a tone that distinguishes between two sounds having similar properties: pitch, loudness and duration (Goldstein, 2010). A pure tone has a shape of a simple wave form with a single component. On the contrary, music instruments have a complex wave signal consisting of a fundamental and several overtones. Since none of the musical instruments produce a simple wave, attention to these components such as fundamental and overtones of complex sound waves result in the perception of timbre (Hodges and Sebald, 2011).

Harmonic Series

Any complex tone having more than a single sine wave contains the frequency that is heard as a pitch of the note along with few other frequencies above it, which is

referred to as a fundamental frequency (Hosken, 2015). For instance, a plucked violin string will begin vibrating at a certain frequency known as fundamental frequency (Tomecek, 2010). The fundamental frequency will have the greatest amplitude in the spectrum and is the most prominent frequency as well. It is this fundamental frequency which is responsible for the perceived pitch of a tone (Meyer, 2009). The individual sine wave that make up a complex tone is referred to as partials. It is named after its partial characterization of making up the complex tone. In this manner, the lowest pitched partial is the fundamental frequency. Tones other than fundamental are generally pitched higher and are referred to as overtones (Loy, 2006). However, it should be noted, that both fundamental and overtones are partials. In like manner, the frequency component of a sound that are whole number multiples of the fundamental are harmonics. These harmonics adds up to the peculiarity of fundamental frequency in any individual sound. Similarly, those components of the spectrum that are not proportional to the fundamental frequency make up the overtones (Moylan, 2015). As a matter of fact, the term harmonic is equivocal. It could refer to frequencies above the fundamental including the fundamental, the first harmonic, the second harmonic and so on. Also, it could include fundamental as its first harmonic making the series first harmonic (fundamental), second harmonic, third harmonic and so forth (Hosken, 2015). Elaborating further, Hosken (2015) adds, "Overtone series would consist of fundamental frequency plus the first overtone, the second overtone etc." (p. 37). The melody and harmony in a musical piece is usually carried by instruments having harmonic partials. It is because the frequencies of the harmonics tend to be in tune along the frequency with the pitches of the diatonic scale. Since, the frequencies of the instruments with inharmonic partials (such as bells and drums) are not in tune with the diatonic scale, they usually don't carry melody and harmony in a musical piece (Loy, 2006). For any given instrument or voice, there are certain frequencies within the spectrum that are emphasized consistently irrespective of the fundamental frequency. The tones within that region all receive the same tone color. The change in amplitude in these tones does not change their frequencies with change in pitch. These areas are called formants (Moylan, 2015) (Meyer, 2009).

CONCLUSION

Possibly, music is hardwired into human brain (Masataka, 2007) (Bennet and Bennet, 2008) (Peretz, 2002). This exceptional ability of transformation from sound waves to neural impulses gives rise to the musical experience (Seashore, 1967). As mentioned above, the conversion of acoustic wave energy into electro-chemical energy (or nerve impulse) called transduction (Tan *et al.*, 2010) is rarely as simple as it seems, and it is the specificities and variances of this conversion that can result in various aural impacts on listeners. There are strong ongoing traditions of research in disciplines such as music psychology, psychoacoustics, systematic musicology and other areas to study the impacts of sounds on humans, including research that contemporary societies use to justify the importance of music. This study has made an effort to provide a preliminary understanding of psychoacoustics properties of sound indicating towards the existing interrelation of these four properties. Future studies may explore the effects of pitch on other properties of sound, as it is one of the crucial sonic property.

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HOW WOULD BE OUR GREAT - - - GREAT GREAT SONS AND DAUGHTERS LOOKING IN REMOTE FUTURE?

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So far the existence of life is concerned; at least our earth is the only planet of solar system where living beings are found in highly diversified form. Since our universe is very vast, it has millions of planetary clusters like our own; we can expect many more celestial platforms where life would definitely be flourishing. Since the time of origin of life on our planet, unimaginable types of variations occurred that resulted into differentiation of varied types of life, i.e., from single cell to multi-cellular types. Things like buried rocks, hard calcified biological materials to skeletal remains have been used as evidences to know the organic world that existed many millions years ago. In the long 3.5 billion years of organic history of evolution, we *Homo sapiens* are the finest product of this natural phenomenon. Our origin is anticipated about 5 million years ago from a common ancestor that gave rise to Chimpanzee and humans. In fact, during the entire journey of biological evolution, we have spent very little period on this planet, however, our extraordinary mental capability has helped us to understand the past and future happenings of major evolutionary events.

Those who understand evolutionary events do think curiously about their future generations, particularly, the major changes in their external appearance, intelligence, technological advances and physical ability. Evolutionary changes are ever going process. This influences every sort of life, whether microscopic organisms through giant mammals. We are, in fact, the consequence of evolutionary changes that has made us to prosper since the dawn of our origin. While advocating evolution of our own race, we talk about the existence of human species that existed from *Ramapithecus* to *Australopithecus* to *Homo habilis* to *Homo erectus* to *Homo sapiens*. Our children, while studying aspects of human evolution in their text books take this topic humorously when they see the pictures of different ancient human descendents. Such pictures are almost real as they have been built on the skeletal remains of our age old ancestors. The fossil remains obtained from the caves and from excavations are in fact subjected to radioactive dating which help us to know the age of such

fossils and then, a picture appearance of the fossils give idea about the physical look of the individuals existing during those days. It is quite likely to think about our past and future. Regarding remote past, we try to accumulate scientific evidences to articulate a real story about our history. For future, may be for 500 or 1000 or 10,000 years or even more from now, only probable anticipation can be made which may be supported by some philosophical clues.

What distinct changes are likely to occur in the purview of physical appearance, physical and mental ability, family bonding and religious believes? Since evolution is an unstoppable process, the evolutionary changes in our own species are inevitable. Mutations in our gene pool would result into new traits. The evolutionary forces which result to change in gene frequency would cause a substantial change in the genetic makeup of our remote future populations. Some of the probable changes could be: Larger size of cranium accommodating more developed brain. Since the origin of humans, steady increase in the cranial capacity has been recorded. This trend is expected to occur due to directional selection. The future populations would be fairer due to loss of hairs. In humans, a number of morphological traits are rudimentary, which persisted in better developed structures in other mammals or animals from which we are expected to originate. The reduction in the size of such structures might have initiated in ancient time due to mutational changes in the genes determining their appearance. Such changes, in fact, did not influence the functioning and survival of its race and therefore allowed to happen. To answer that in future descendents, some more organs may undergo severe modification, cannot be ruled out. Based on this concept, one can expect substantial changes in the overall look of human face. Due to differences in the feeding materials and feeding habits, chin will recede and become shorter.

A remote future descendents would have most notable change in their skin colour. Due to day activity being confined to shelter places, the skin will get fairer. We are witnessing hectic activities going in the indoor places or people using skin protecting creams or cover throughout the

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world; it would leave its effect on the concentration and distribution of colour cells in our skin. Some believe that our future descendents would have higher occurrence of myopia (short sightedness) and allergies problems. Reasons for this have been suggested due to increasing optical activities requiring more stress and strain over iris muscles, cases of myopia will have higher frequency in the population and people will have allergic reactions more common due to their more hygienic living habits, consequently, they would not be exposed to varied kinds of allergens. Primates like gorilla, chimp or other apes have long pointed canines. Since the onset of humans, a steady decrease in the size of canines has been observed. Based on the feeding habits it is quite likely that our future races would have much degraded canines. Overall strength of our bite would be weaker due to our habituation to feed soft food materials. Some people even predict disappearance of little toes.

Whether in remote future, one can expect existence of two separate species of humans? To my consideration, it is a remote possibility or straight way, it can be said that no two species of humans will exist together on this planet. Due to globalization, mixing of different human races have enhanced in the present era than before and therefore speciation by splitting our existing species into two and being separated for a long period that no gene flow occur between them seems to be impossible. This may only happen if a separate population of us inhabit a new space (planet) and remain cutoff for a period that cause enough genetic variation leading to two distinct gene pools that refrain gene flow. It is scientifically documented that once upon a time, we lived with Neanderthal, but what really resulted the complete wiping out of *Neanderthal*, is one of the major puzzle that we answer by saying that intense fighting between Neanderthals and *Homo sapiens sapiens* resulted their elimination. Existing two wise communities on the same land simultaneously with almost similar needs would definitely cause intense conflict.

The growing intelligence of future human beings will make them evolve technologically, to which one can refer digitally. Their reliance on artificial intelligence will dominate than their relations. Everyone will have one's DNA profile which will assist physician to treat the person according to his or her individual characteristics. The field of nanotechnology would be assisting in the treatment of enumerable ailments including cancer. Transgenic

organisms will be providing bizarre organic products for human utilization. Space would be one of the highly utilized spot from where a number of our activities at the ground will be monitored. Access to planetary bodies, particularly, mars will be most preferred endeavor for bringing raw materials from there to ground for our use. Advancement in the field of medical sciences would be unbelievable, especially with respect to organ transplant and stem cell therapy. Treatment of infectious diseases would be ever challenging due to evolution of highly resistant microorganisms, but unprecedented research, would help to overcome such challenges. Our own activity may harm us tremendously. Man- made disasters may result into global destructions resulting into nuclear winter and the survivors may have unique adaptability to nuclear radiations which can alter our lives drastically. There would be 100 percent literacy and people will have total faith in scientific advances that would lead to least affiliation for religious trust, superstitions and age old cultures. Very unexpected changes will be there with respect to family cohesion. This world has seen mass living of human community during ancient time period for mutual safety and immediate gain. Large combined families split into nuclear families in quite wide spread parts of this globe. Psychological set up of existing community indicates further dissection in the family forms leading to individuals living with profuse promiscuity in both sexes. Whether more distinct changes will appear between the two sexes of our species or sexual dimorphisms will be unaltered in long course of our evolutionary journey? Darwin's natural selection shapes the morphological forms of animals. Natural selection in the form of sexual selection has been one of the major factors in the expression of differential sexual features in the two sexes of majority of animal species. Reduction in average height of females than their male counterpart in majority of human races is the result of this selection. Preferential mating in humans based on body size variation will maintain this gap and the difference even may lead to more significant differences. As a consequence of this, females in remote future may show significantly shorter height compared to the male community.

A politician in India, once raised a question saying that how can a monkey change into a human being as claimed by evolution biologists. He said that none of us has seen anywhere in the forest or at any spot changing a monkey into a man. He refuted Darwin's theory that

advocates evolutionary events. Remarks made by the minister triggered a wave of discussion by news and television media to counter minister's statement. In fact, understanding the ways by which evolutionary events take place, to see a species changing into another one needs lot of scientific knowledge. Such things can now be explained authentically, i.e., based on scientific evidences. The fossil remains are the direct evidences of human species or races that persisted in remote past and were much different from the present day humans. We have been able to articulate scientific history of our own species and also other vertebrates whose fossil remains have been obtained in

better conditions. The process of evolution is extremely slow. To observe obvious changes in a species from its remote ancestral species needs millions of years of time span. How a monkey gave rise to an ape and a form of ape gave rise to a human race, needs understanding of the phenomenon of geographical isolation, changes in the genome by mutation and chromosomal rearrangement, reproductive isolation etc., and all these take a long period to occur. It can be, at the end said with complete certainty that such issues would not be the contentious one, once people would be imbibed with scientific knowledge of evolutionary events.

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