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RESEARCH ARTICLE

EFFECT OF TYRE SMOKE EXPOSURE ON SOME HAEMATOLOGICAL PARAMETERS OF SELECTED ABATTOIR WORKERS: A TRANS-AMADI (OGINIGBA TOWN) SLAUGHTER MARKET STUDY

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Abstract

This investigation was conducted to study the effect of exposure to tyre smoke on serum calcium (Ca) and albumin (Alb) of selected abattoir workers in Oginigba area of Obio Akpor L.G.A., Rivers State. A total of 50 participants were recruited for the study (35 test and 15 control). The test population was divided into five study groups; 1-3, 4-6, 7-9, 10-15 and above 15 years of service (exposure years) at the abattoir. Blood samples were collected by veinupuncture and analyzed for serum calcium and albumin using the colorimetric and bromocresol-green method respectively. Compared to 3% of the control population, 49% of the test population had a serum calcium level below the standard reference of 2.20-2.55mmol/l. Results of serum albumin showed that 23% of the test population had levels above the standard reference of 3.5-5.0g/dl whereas the control population had values within the reference range. When the respective test groups were compared with the control, groups 1-3, 7-9 and 10-15 showed significant reduction in serum calcium level while groups 7-9 and 10-15 showed significant increase in the serum albumin level. Serum calcium was observed to significantly differ from the control at the least level of exposure (1-3 years) while serum albumin differed significantly from the control at 7 years of exposure for non-smokers and 4 years of exposure amongst smokers. The data was also grouped into two study groups based on genotypes AA and AS. It was observed that participants with genotype AS were more susceptible to the effect of the smoke inhalation and recorded low serum calcium level. The result of this research has demonstrated that abattoir workers (roasters) are prone to developing hypocalcaemia and hyperalbuminaemia, and as such require periodic medical attention, care and better animal processing (dehairing) practice/method.

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Introduction:-

Toxic chemicals pose potential health risks and when present at operational areas can affect productivity. Some exposures are dangerous and even lethal. These compounds cause harm by varied mechanisms and the extent of injury vary widely depending on the degree of exposure and on the biochemical properties of the inhalant.

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Tyre combustion produces particulate matters which are aggregates of small particulates, liquid droplets and vapours. The particulates are of aerodynamic diameter $PM_{2.5}$ and PM_{10}. Tyre combustion results into volatiles which undergo degradation to produce free radicals considered biologically hazardous (Bosire et al., 2016). These can represent both significant short term and long term hazards to individuals and the environment (Tsai, 2016). Emissions from burning of tyres are associated with serious public health ailments including whizzing of the lungs and chronic coughs. Emissions from burning tyres are highly mutagenic and carcinogenic (Downard et al., 2015). Open tyre fire emissions are estimated to be 16 times more mutagenic than residential wood combustion in a fire place and 13000 times more mutagenic than coal fired utility emission with good combustion efficiency and add-on controls (Reisman, 1997).

In most rural abattoir in Nigeria and particularly in Trans-Amadi (Oginigba Town) slaughter market, Obio/Akpor Local Government Area (LGA) of Rivers State, the dehairing of slaughtered animals is often carried out in open fire points using old tyre scraps or woods as main fuel source. The toxic nature of open tyre burning and its emissions of toxins inhaled by abattoir dehairing staff (roasters), poses significant acute and chronic health hazards, depending on the duration of exposure.

The need to investigate and evaluate the adverse effect of exposure and inhalation of these toxins from tyre burning for the purpose of dehairing by abattoir workers on selected haematological parameters is imperative.

Calcium, an abundant mineral present in the human body of normal healthy adults provides structural support to the skeleton (Bolarin, 2013). They are mostly protein-bound within cell membrane, mitochondria and endoplasmic reticulum which are localized in the intracellular space. Cell membrane and organelles regulate calcium levels through an active transport pumps and second messenger system which can be impeded xenobiotics or some toxic substances (Nilus, Schwarz and Droogmans, 1993).

Albumin as an important and abundant blood serum protein primarily synthesized by the hepatocyte accounts for about 55-60% of estimated serum protein and an important prognostic indicator, as low serum levels is indicative of high risk of morbidity and mortality (Gosling, 1995; Kerl and Cohn, 2004; Peralta and Rubery, 2017). Its synthesis is dependent upon osmotic environment, nutritional, hormonal and disease states. Since serum albumin is an important health indicator, it is used in a wide range of settings like diagnosis of disease; monitoring health status changes upon treatment or disease progression; and screening to ascertain inclusion of other forms of testing. Some severe stages hypoalbuminemia may consequently lead to notable and bizaar life-threatening conditions such as muscle fatigue, inflammation, appetite loss, prolonged wound healing and tissue repair and pleural effusions (Doweiko and Nompleggi, 1991).

Report by Bosire et al. (2016) states that burning of tyres gives off numerous volatile substances which upon degradation produce free radicals which are hazardous biologically. Tyre burning emits pollutants which are harmful, toxic, mutagenic and carcinogenic to humans and animals (Downard et al., 2015).

However, the intensity and extent of exposure to scrap tyres burning could pose a direct adverse health effects on humans and these may include: respiratory complications (asthma), cough and chest pain, cardiovascular illnesses, eyes irritation, tumor, inflammation of mucous membranes, irregular blood pressure and ensuing organ diseases (Chein et al., 2003).

Methodology:-

Materials and reagents:

Kits, chemicals and reagents used for this study were all procured from accredited outlets and distributor, and of analytical grade.

Study population and size:

A total of 50 volunteers consisting of thirty-five (35) test (abattoir workers) and fifteen (15) control (non- abattoir workers) participated in this study. Both the test and control population were inhabitants of Port Harcourt, Rivers State. The test population was selected from the carcass handlers (roasters) at the abattoir while the control population was selected outside the abattoir.

Sample collection and preparation:
Procedure:
Each participant's name, age, sex, length of service at the abattoir, possible health challenge and smoking habit were noted and sample bottles appropriately labelled. Whole blood sample is collected from each participant via venupuncture and strict adherence with standard guideline for blood sample collection.

The tourniquet is placed on the arm about four inches above the vein of interest, and the area thoroughly cleaned and disinfected with 70% alcohol by at least 2cm by 2cm for 1 minute and allowed to dry. The vein is then anchored to prevent blood from rolling by pulling the skin below the vein taut and a needle with syringe inserted at a 15 to 30˚ angle and held still. Once blood flow is initiated, tourniquet is released and after the required volume of blood has been drawn, the needle is gently removed and the site of puncture covered with a dry cotton wool with a mild pressure. The sample collected is transferred immediately into a plain vacutainer bottles for laboratory analysis. At the laboratory, the blood is allowed to clot, dislodged and centrifuged at 5000rpm for 10 minutes. The supernatant (serum) is transferred immediately into a clean polypropylene tube using a pipette and maintained at 2-8˚C before use.

Calcium Estimation: Colourimetric Method:
Procedure:
Three (3) test tubes were labelled (reagent blank, standard and sample) and used for the assay. To the test tube labelled reagent blank, 25µl of distilled water was introduced to it; the standard test tube had 25µl of calcium standard introduced into it; and to the sample test tube was introduced 25µl of test sample (serum). Solutions R1 and R2 (0.5ml each) was added to the three (3) test tubes, mixed properly and incubated at 20 - 25^{o}C for 50 minutes. Absorbance reading of sample and standard against the reagent blank was done in a spectrophotometer at a wavelength of 570nm and sample value extrapolated thus;

Concentration (mg/dl) = $A_{sample} \div A_{standard} \times$ Standard concentration (mg/dl)

Note: Calcium standard concentration is 2.55mmol/l

Albumin Estimation: Bromocresol-Green:
Procedure:
Three (3) test tubes were labelled (reagent blank, standard and sample) and used for this assay. To test tube 1 (reagent blank), 0.5ml of distilled water was introduced; test tube 2 (standard) had 0.5ml albumin standard introduced to it; and 0.5ml of sample (serum) was introduced to test tube 3 (test). 3.0ml of solution R1 was added to the three (3) test tubes, properly mixed and incubated for 5 minutes at 20 - 25˚C. Absorbance reading of sample and standard against the reagent blank was done in a spectrophotometer at a wavelength of 630nm and sample value extrapolated thus;

Concentration (g/dl) = $A_{sample} \div A_{standard} \times$ Standard concentration (g/dl)
Note: Albumin standard concentration used is 4.64g/dl

Haemoglobin Electrophoresis:
Procedure:
The cellulose acetate paper is soaked in buffered water (pH 8.6), then blotted dry with Whatman filter paper. 100ml of buffer is poured into each of the outer sections of the electrophoretic chamber, with two (2) Whatman filter papers wrapped over each support bridge to ensure contact is made with the buffer. The haemolysate is prepared by centrifuging the sample, washing the red cells with normal saline (thrice) and then lysing cells with distilled water. The haemolysate is applied on the blot dried cellulose acetate paper with the aid of an applicator, and the cellulose acetate paper transferred immediately to the electrophoretic chamber with each ends placed on wet Whatsman filter paper making contact with the buffer. The power source is turned on and the lid of the machine properly covered. Electrophoresis of the sample is allowed for 20 minutes and the result interpreted thereafter from the pattern of separation.

Result and Discussion:-

Serum calcium and albumin estimation of the control population:

Table 1 reveals the test results of serum calcium and albumin levels from 15 healthy, none smoking male participants who do not live or work around the abattoir and its environs.

Given the standard reference range for normal serum calcium as 2.20 - 2.55mmol/l, 1 (3%) out of the 15 control participants fell below the reference range and 14 (97%) out of the 15 total control participants has a calcium content within the reference range.

From normal serum albumin reference range of 3.5-5.0mg/dl, all 15 participants have albumin content within reference range (i.e. 100% of values are within reference range) as expressed in table 1.

Table 1:- Serum albumin and calcium concentration of the control population.

S/N	Calcium (mmol/l)	Albumin (g/dl)	Age (years)
1	2.29±0.01	4.71±0.00	30
2	2.29±0.00	4.72±0.01	24
3	2.19±0.00	4.66±0.01	38
4	2.23±0.02	4.55±0.00	20
5	2.45±0.01	4.75±0.01	23
6	2.37±0.00	4.69±0.00	30
7	2.40±0.00	4.71±0.00	37
8	2.35±0.01	4.40±0.01	29
9	2.50±0.00	4.59±0.00	24
10	2.32±0.03	4.57±0.00	34
11	2.09±0.01	4.55±0.00	20
12	2.16±0.00	4.58±0.01	22
13	2.37±0.00	4.77±0.00	31
14	2.28±0.00	4.68±0.00	26
15	2.34±0.00	4.56±0.00	33

Data are presented as mean ± standard deviation (SD) of triplicate evaluation.

Serum calcium and albumin estimation of the test population:

The mean results of serum calcium and albumin estimation of 35 healthy participants selected from the abattoir of the 'roasters' community were reported in table 2. The table (2) shows the code number, age, level of exposure and smoking status of the test (study) population. The values from analysis of calcium and albumin of the test population are also stated.

The reference range for normal serum calcium is 8.8-10.2mg/dl equivalent to 2.20-2.55mmol/l (Stephen and Karen, 2006). From the total test population of 35 participants, 18 of 35 (51%) are within the reference range and 17 of 35 (49%) are below the reference range.

The reference range for normal serum albumin is 3.5-5.0 g/dl (Burtis, Ashwood and Brun, 2012). From the study population of 35 participants, 8 of 35 (23%) are above the reference range (of these, 9% are cigarette smokers) and 27 of 35 (77%) are within the reference range (table 2).

Table 2:- Mean Serum calcium and albumin estimation of the test population.

S/N	Calcium (mmol/l)	Albumin (g/dl)	Genotype	Age (years)	Exposure (years)	Smoker (YES/NO)
1	2.10±0.00	4.58±0.01	AS	38	3	NO
2	2.18±0.00	4.85±0.00	AA	37	3	NO
3	2.21±0.01	4.76±0.00	AA	25	8	YES
4	1.92±0.00	5.03±0.01	AA	26	6	YES
5	2.22±0.00	4.60±0.01	AA	20	3	NO
6	2.15±0.01	4.93±0.00	AA	29	15	NO
7	2.28±0.02	4.68±0.00	AA	23	10	NO

8	2.26±0.00	4.06±0.00	AS	22	4	NO
9	2.27±0.00	5.28±0.02	AA	21	5	NO
10	2.29±0.00	4.56±0.00	AS	20	2	NO
11	2.31±0.01	4.90±0.00	AA	21	4	NO
12	2.37±0.00	5.13±0.00	AA	37	19	NO
13	2.30±0.00	5.02±0.00	AA	40	17	NO
14	2.16±0.03	4.47±0.01	AS	24	4	NO
15	2.04±0.01	4.79±0.01	AS	30	2	NO
16	2.32±0.00	5.15±0.01	AA	40	5	YES
17	1.74±0.03	3.65±0.00	AA	37	5	NO
18	1.81±0.00	4.83±0.03	AA	42	27	NO
19	2.21±0.02	5.35±0.00	AA	40	5	NO
20	2.14±0.01	4.61±0.00	AA	45	7	YES
21	2.42±0.01	4.65±0.01	AS	46	10	NO
22	2.47±0.00	4.57±0.00	AA	27	4	NO
23	1.99±0.03	4.29±0.00	AA	25	2	NO
24	2.11±0.01	4.85±0.00	AS	34	7	NO
25	2.21±0.00	4.90±0.02	AA	45	11	NO
26	2.24±0.02	4.45±0.01	AA	28	4	NO
27	2.02±0.00	4.88±0.00	AS	35	12	NO
28	2.27±0.00	4.47±0.02	AA	29	4	NO
29	1.98±0.01	4.76±0.00	AS	42	11	NO
30	1.79±0.01	4.68±0.00	AS	42	10	NO
31	2.22±0.00	5.12±0.00	AA	39	5	YES
32	2.08±0.00	4.90±0.04	AA	32	9	NO
33	2.12±0.00	4.83±0.01	AA	36	7	NO
34	2.22±0.01	4.68±0.01	AA	39	3	NO
35	1.87±0.00	5.14±0.00	AS	41	10	NO

Data are presented as mean ± standard deviation (SD) of triplicate evaluation.

Correlation of serum calcium levels of the reference range, test and control populations:
With the reference range of normal serum calcium as 2.20-2.55mmol/l (Stephen and Karen, 2006), 17 of the 35 (49%) test (experimental) population fell below the reference range whereas 1 of 15 (3%) from the control population had a calcium level below the reference range (Fig. 1).

The significant ($p<0.05$) reduction in calcium levels could be as a consequence of low calcium and vitamin D absorption reported among smokers (Need et al., 2002). Hypocalcaemia could also have arisen as a result of deficiency in parathyroid hormone (PTH) which can be brought about by a mutation in the calcium–sensing receptors caused by the inhalation of smoke constituents (Skugor, 2014). Noting the consequences of hypocalcaemia which include seizures, impaired intellectual capacity, cardiovascular disease, numbness, muscle spasms, etc, there is a possibility of victims being asymptomatic especially in cases where the conditions develop slowly leading to a fatal stage (Cooper and Gittoes, 2008).

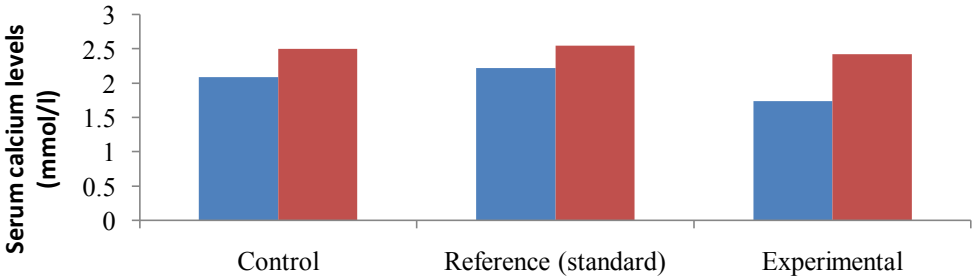

Figure 1:-Serum calcium correlation of the reference range, test and control populations showing the minimum and maximum points for each.

Correlation of serum albumin levels of the reference range, test and control populations:
With the reference range of normal serum albumin given as 3.5-5.0 g/dl (Burtis, Ashwood and Brun, 2012), the control population (15) had serum albumin levels within reference range, whereas 8 of 35 (23%) test (experimental) population had serum albumin levels above the reference range resulting in cases of hyperalbuminaemia (Fig. 2).

Conditions of hyperalbuminaemia are reported to be commonly associated with abrupt dehydration and relatively increased protein diets (Busher, 1990; Mutlu et al., 2006). Irrespective of the antioxidant activity of albumin as a protein, its implication in risk of distal colon cancer has been reported to be high (Knekt et al., 2000).

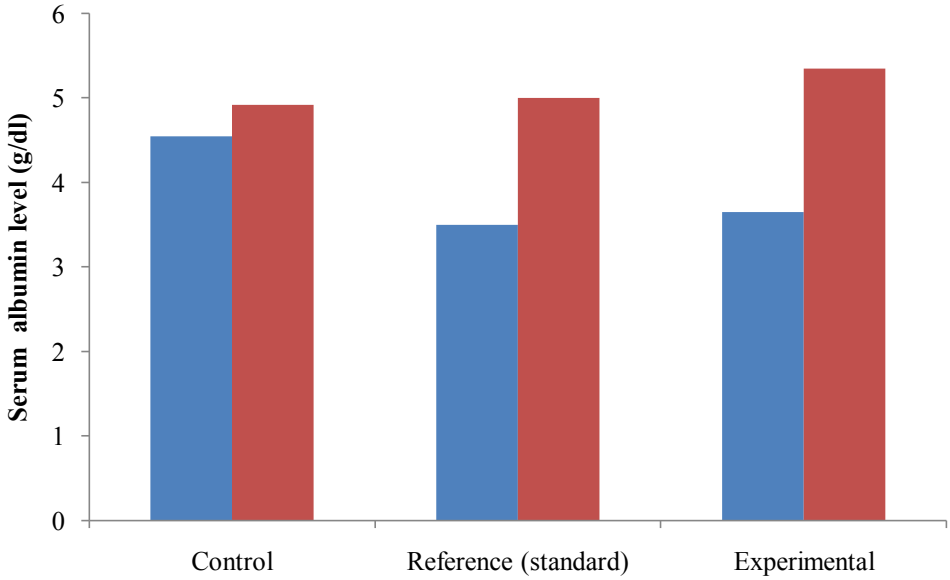

Figure 2:- Serum albumin correlation of the reference range, test and control populations showing the minimum and maximum points for each.

Comparison of the control and the levels of exposure of test (experimental) population:
The experimental population was grouped into five classes based on years of service at the abattoir (1-3, 4-6, 7-9, 10-15 and >16 years). Descriptive statistics and one sample T test were employed to compare the means of the control against the test (experimental) and check for significance at 95% confidence level (table 3).

Table 3:- Mean serum calcium and albumin levels with respect to exposure levels.

Exposure (years)	Calcium (mmol/l)	Albumin (g/dl)
Control	2.31 ± 0.11^{a}	4.63 ± 0.10^{a}
1-3	2.15 ± 0.11^{b}	4.62 ± 0.18^{a}
4-6	2.20 ± 0.19^{ab}	4.70 ± 0.51^{ab}
7-9	2.13 ± 0.05^{b}	4.79 ± 0.11^{b}
10-15	2.06 ± 0.17^{b}	4.83 ± 0.17^{b}
>16	2.16 ± 0.31^{ab}	4.99 ± 0.15^{a}

Values are means ± SD of triplicate evaluation. Values in a column with different superscript are significantly different ($P<0.05$).

Serum calcium comparison of control and levels of exposure (years):
From the statistical analysis of serum calcium levels, the control differs significantly ($p<0.05$) compared to groups 1-3, 7-9 and 10-15 years of exposure. This implies that at the least exposure (1-3), there is a significant difference in serum calcium level. However, there was no significant difference ($p>0.05$) when years of exposure was compared among the different levels (Fig. 3).

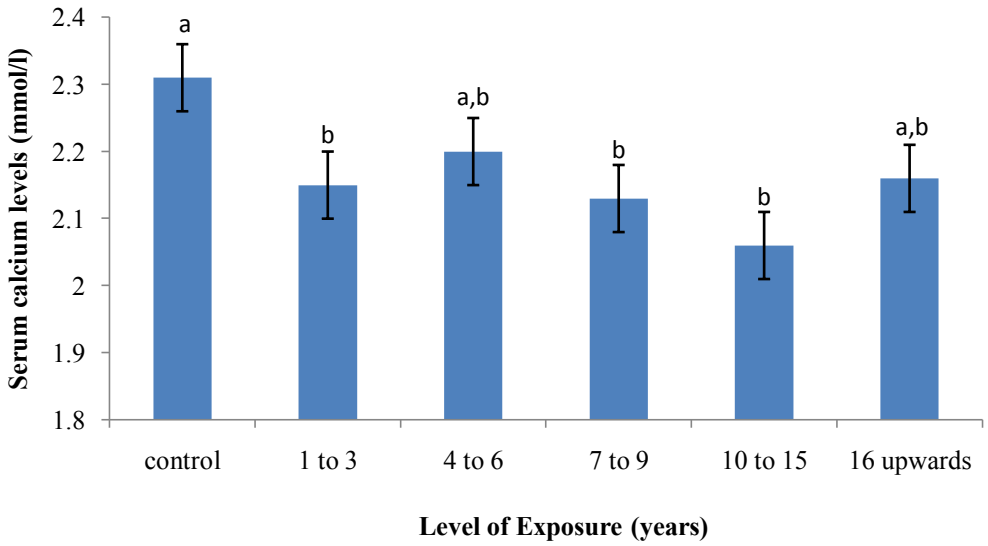

Figure 3:-Mean Serum calcium levels at various exposure years. Bars with different alphabets are significantly different ($p<0.05$) compared to control.

Serum albumin (g/dL) comparison of control and levels of exposure (years):
From the statistical analysis for serum albumin, the control differs significantly ($p<0.05$) from those with 7-9 and 10-15 years of exposure respectively (fig. 4). This implies that at 7-9 years of exposure, the difference is significantly evident. On the contrary, there was no observed significant difference ($p>0.05$) existing between the control and exposed (1-3, 4-6 and >16). Therefore, it could be said that those at 1-3 and 4-6 years level of exposure suffered minimal impact and are said to be safe from the adverse effect of increased serum albumin levels as a result of smoke inhalation.

However, it is worthy of note that given the standard reference range of serum albumin to be 3.5 to 5.0 mg/dl, of the 12 participants making up 4-6 years of exposure, 5 (42%) actually had a higher serum albumin level, especially among the cigarette smokers. Hence, the result reveals that those with of 1-3 years of exposure and are none smokers seem still safe from the effect of burnt tyre smoke inhalation as indicated by serum albumin levels.

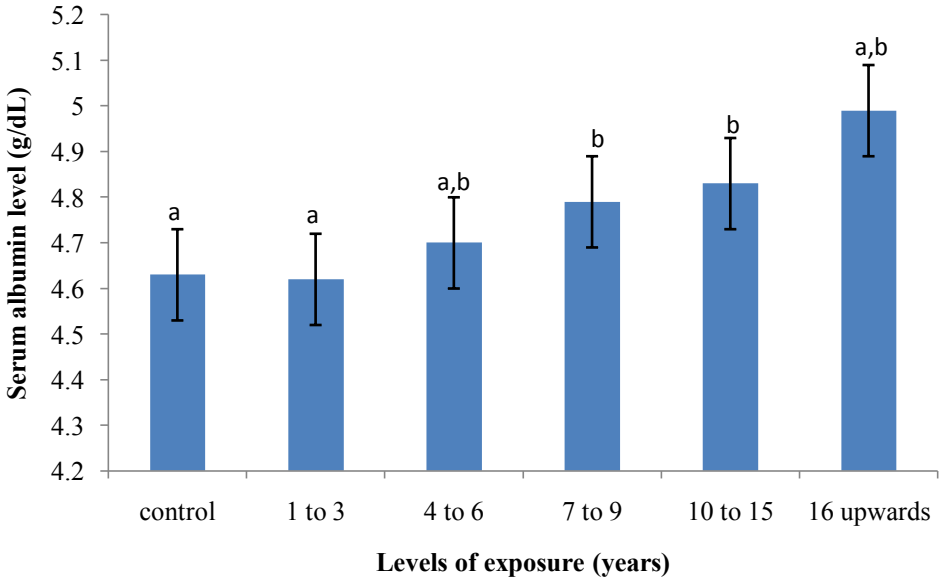

Figure 4:-Mean serum albumin levels of exposure. Bars with different alphabets are significantly different ($p<0.05$) compared to control.

Correlation of calcium with albumin levels:
The investigation revealed reduced serum calcium levels in 49% of test (experimental) population but no hypoalbuminaemia was recorded. This is not in line with several reports on calcium and albumin relationship. Approximately 40% of total plasma calcium circulates bound to albumin. When circulating albumin is reduced, total serum calcium must be adjusted to reflect low amounts of bound calcium. More specifically, each 1g/dL decrease in albumin concentration will result in 0.8mmol/l decrease in serum calcium. Thus, 0.8mmol/l must be added to the measured calcium to obtain an actual calcium value (Fox, 2017).

Inhalation of burnt tyre and its constituents by abattoir workers results in parathyroid hormone (PTH) deficiency brought by mutation in calcium-sensing receptor and is a major cause of hypocalcaemia (Skugor, 2014).

Genotype based comparism among participants:
The test (experimental) population of 35 participants was grouped into two genotype classes (AA and AS), and 24 participants were of genotype AA while 11 participants were of genotype AS (table 4).

Given that the standard reference calcium level ranges from 2.20-2.55mmol/l (Stephen and Karen, 2006), 9 (37.5%) out of the 24 test participants with genotype AA had serum calcium levels below the reference range, whereas 8 (72.7%) of the 11 test population participants with genotype AS had serum calcium level below normal. It can therefore be deduced that participants with genotype AS were more susceptible to the effect of burnt tyre smoke inhalation resulting in diminishing serum calcium levels.

For serum albumin, with the standard reference range of 3.5-5.0g/dl as stated by Burtis, Ashwood and Brun, (2012), 7 (29.2%) out of the 24 test participants with genotype AA had serum albumin level above reference range whereas 1 (9.1%) of the 11 test participants with genotype AS had serum albumin above normal reference range. It can therefore be deduced that participants with genotype AS were less susceptible to the effect of the smoke inhalation on increasing albumin levels (table 4).

Earlier research involving sickle cell trait (genotype AS) revealed that sickle cell trait has a protective advantage against plasmodium falciparum, although with associated rare complications (Williams et al., 2005). From the reports of Judd et al., (2003), elevation in intracellular calcium levels and alterations in its cellular sensitivity were found to be associated with sickle cell disease (SCD) and erythrocytes trait (AS). Literature explaining the mechanism of burnt tyre smoke inhalation on serum calcium levels leading to increased susceptibility of participants with sickle cell trait to low calcium levels as observed in this study is absent.

Table 4:- Serum calcium and albumin concentration with respect to genotype.

GENOTYPE				
S/N	AA		AS	
	Calcium (mmol/l)	Albumin (g/dl)	Calcium (mmol/l)	Albumin (g/dl)
1.	2.18±0.00	4.85±0.00	2.10±0.00	4.58±0.01
2.	2.21±0.01	4.76±0.00	2.26±0.00	4.06±0.00
3.	1.92±0.00	5.03±0.01	2.29±0.00	4.56±0.00
4.	2.22±0.00	4.60±0.01	2.16±0.03	4.47±0.01
5.	2.15±0.01	4.93±0.00	2.04±0.01	4.79±0.01
6.	2.28±0.02	4.68±0.00	2.42±0.01	4.65±0.01
7.	2.27±0.00	5.28±0.02	2.11±0.01	4.85±0.00
8.	2.31±0.01	4.90±0.00	2.02±0.00	4.88±0.00
9.	2.37±0.00	5.13±0.00	1.98±0.01	4.76±0.00
10.	2.30±0.00	5.02±0.00	1.79±0.01	4.68±0.00
11.	2.32±0.00	5.15±0.01	1.87±0.00	5.14±0.00
12.	1.74±0.03	3.65±0.00		
13.	1.81±0.00	4.83±0.03		
14.	2.21±0.02	5.35±0.00		
15.	2.14±0.01	4.61±0.00		
16.	2.47±0.00	4.57±0.00		

17.	1.99±0.03	4.29±0.00		
18.	2.21±0.00	4.90±0.02		
19.	2.24±0.02	4.45±0.01		
20.	2.27±0.00	4.47±0.02		
21.	2.22±0.00	5.12±0.00		
22.	2.08±0.00	4.90±0.04		
23.	2.12±0.00	4.83±0.01		
24.	2.22±0.01	4.68±0.01		

Data are presented as mean ± standard deviation (SD) of triplicate evaluation.

Conclusion:-

Exposure to burning tyre or wood smoke has been shown to affect individuals' health in various ways (Larson et al., 2007; Le-Van et al., 2006). The research findings are evident that some of the roasters (singeing) are in dire need of medical examination and attention, as results indicates that 49% of the test participants had serum calcium level normal reference range while 23% had serum albumin levels above normal reference range.

Safe exposure limit to the fire points could not be established as the serum calcium level differed significantly with the control at the least years of exposure (1-3 years) used in the study while serum albumin levels differed significantly from the control from 7-9 years of exposure for none smokers and 4-6 years of exposure among cigarette smokers.

In addition to the effect of inhaling burning tyre smoke at the abattoir, decreased serum calcium levels (hypocalcaemia) was observed to be positively influenced by genotype AS while increased serum albumin levels (hyperalbuminaemia) was observed to be positively influenced by cigarette smoking.

The study conducted clearly indicates that the effect of inhalation of smoke particles from burning of tyres lowered serum calcium levels which could possibly lead to increased calcium mobilisation thereby predisposing the affected participants to osteoporosis.

Conflict of Interest:

Authors state that there was no conflict of interest in the course of this research study.

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