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FRESHWATER RESOURCES COULD BECOME THE MOST CRITICAL FACTOR IN THE FUTURE OF THE EARTH

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ABSTRACT

Freshwater resources could become the most valuable commodity in the world in the near future. Freshwater can be defined as water that contains less than 1,000 milligrams per liter of dissolved solids. Even though, freshwater constitutes only 2% of total global water, it is essential for the existence of all terrestrial and freshwater lives, as well as marine lives. Increased demand and the gradual shrinking of water tables could lead to freshwater shortages. If temperatures increase and pollution of freshwater continues, this would further deplete available freshwater. Ultimately, if human populations on Earth expand as they have in recent times, then freshwater will at a premium and will require proper management to safeguard not only human health, but the quality of the entire global ecosystem. This could require desalination of sea water in order to have potable and usable water. Although desalination is currently cost prohibitive, this may become necessary due to lack of other alternatives. This paper will discuss the impacts of overpopulation and possible climate change on the distribution and accessibility of fresh water, and how freshwater scarcity may affect the lives on earth.

Key words: Overpopulation, climate change, freshwater resources, freshwater shortage.

INTRODUCTION

Fresh water is a precious resource. It is necessary for sustaining all forms of terrestrial life on Earth. Clean drinking water is essential to support human health. Freshwater is also needed for agricultural and manufacturing activities, inland navigation, etc. Such usage can put significant stress on existing water resources, especially when demand is high and supply is low. In many areas, such stress can be more significant due to a sharp rise in human population and changing climatic conditions such as melting mountain snow and glaciers, rising sea levels, frequent droughts, forest fires, and severe weather conditions.

Freshwater can be defined as water that contains less than 1,000 milligrams per liter of dissolved solids, most often salts (USGS, 2016a). The water cycle includes freshwater bodies as renewable resources, but the cycle is also very much dependent on-salt water resources. The amount of freshwater in waterbodies is ever-changing due to constant inflows (from runoffs and precipitation, among others) and outflows (evaporation, underground seepage, consumption and usage, etc.). The earth adapts different hydrologic conditions with the ever-changing weather pattern. For instance, during the last ice age, snow, ice and glaciers covered much more land surface than today and glaciers contributed to water contained in the great lakes, which is about 20% percent of the total available freshwater on planet Earth (USGS, 2016a).

Freshwater is scarce in some regions, countries, or even continents (Berger and Finkbeiner, 2010). The estimated water requirements for meeting basic human needs is 50 liters per person per day (Gleick, 1996). However, according to a 2003 report by the World Health Organization (WHO), about 1.2 billion people do not have access to safe and affordable water for their domestic use (WHO, 2003). World Bank Group (WBG) has reported that currently, about 1.6 billion people live in regions with an absolute water scarcity. WBG is estimating that it will rise to 2.8 billion people by 2025 (WBG, 2016).

Increased demand and gradual shrinking of water tables will make fresh water the most valuable commodity of the future world. Therefore, it needs to be managed properly to safeguard human health and ecosystem quality. This paper will mostly discuss the availability and distribution of freshwater and the overall effects of changing climates on fresh water resources and how that can affect global ecosystems and human lives.

GLOBAL FRESHWATER CYCLE

Useable fresh surface water is relatively scarce. A very small percentage of freshwater is available to sustain all terrestrial and freshwater life forms. To understand the freshwater availability, let us first review Earth’s total water content, sources and distribution. The estimated total global water content is about 332,500,000 cubic miles; of that the total global fresh water content is only about 8,404,000 cubic miles, the rest is salt water. Freshwater content in lakes and swamps is about 24,600 cubic miles, and in rivers is about 509 cubic miles (Gleick, 1996). In another estimate, about 98% of water of the planet earth is salty and only 2% is fresh. Of that 2% that is freshwater, about 70% is frozen as snow or ice, 30% is groundwater, 0.5% is surface water (rivers, lakes, swamps, etc.) and 0.05% is as atmospheric water (Mcintyre, 2012). According to United States Geological Survey (USGS) about 20% of this surface water in contained in Lake Baikal in Asia and about 20% in the Great Lakes of North America. Rivers contain about 0.006% of total freshwater reserve (USGS, 2016a).

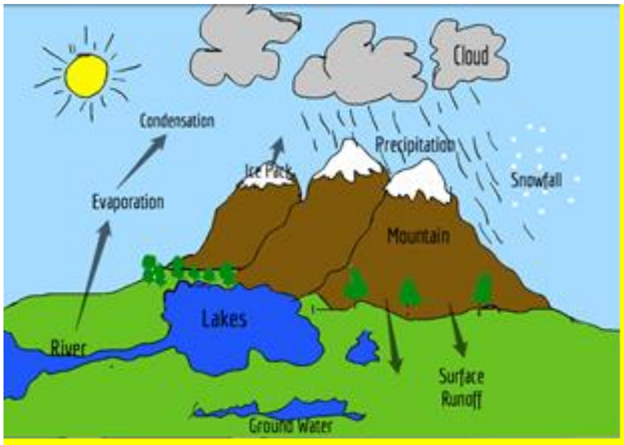


Figure 1. An illustration depicting freshwater cycle of the earth. Even though, freshwater constitutes only 2% of total global water, it is essential for the existence of all terrestrial and freshwater lives, as well as marine lives.

Figure 1 represents a simple view of the freshwater cycle of the earth. This water cycle is also playing a significant role in sustaining marine lives. Without freshwater to replenish water that evaporates from the oceans, ocean water will be too saline to support marine lives.

INCREASED TEMPERATURE AND ITS EFFECTS ON WATER RESOURCES

The concept of climate change has become a highly controversial and political subject. Although this subject will not be debated in this article we can address the consequences if temperatures do increase.

Agriculture and food supply are very much dependent on climate and water supply, and any imbalance in these would affect food production. If the earth’s temperature does rise, it could affect terrestrial water cycles by increased evaporation and droughts and thus will affect food production. The US has experienced severe droughts in recent years (GRACE, 2016).

Snowmelt and mountain runoff are the major sources of fresh water supply. Mountains provide more than 50 percent of earth’s fresh water (GRACE, 2016). Seasonal melting of snow and ice from mountain tops slowly release water into the environment. Warmer temperature will result in less ice on mountain tops, less snow falls, more water evaporation, and thus more rainfalls (GRACE, 2016). Studies indicate that over the 20th century, precipitation has increased by 5 to 10 percent and this trend is expected to continue (Adams and Peck, 2008). Since rain water flows faster than melting snow, this excess water runoff will neither help in recharging ground water tables nor moisturizing deeper soils. This will cause ground water shortages in areas that mostly rely on melting snow as their primary freshwater source.

EFFECTS OF OVERPOPULATION AND POLLUTION ON WATER RESOURCES

Managing freshwater resources is vital for terrestrial ecosystems and human survival. Clean drinking water is important for human health. Sustainable agriculture and manufacturing activities depend on freshwater supply. Increasing usage of fresh water can put significant stresses on existing water resources.

The human population of Earth has increased rapidly in recent times. The UN has calculated that 200 years ago, humans totaled less than 1 billion but this has expanded to more than 7 billion living humans today (Ortiz-Ospina and Roser, 2016). The maximum carrying capacity of Earth is thought to be 9 or 10 billion humans and is based on calculations taking into consideration available resources (Wolchover, 2011).

One major factor in these calculations is that it includes unsustainable resources. For example, aquifers on Earth are being utilized 3.5 times faster than they can be recharged by rainfall (Gleeson, et al., 2012).

Most assessments of global water resources have been focused on surface water (Giordana, 2009; Postel et. al., 1996; Vorosmarty et. al., 2000; Oki and Kanae, 2006). However, groundwater is an essential part of irrigation and maintaining ecosystems. Since most of the freshwater is contained as groundwater, sustainable depletion of groundwater will play a vital role in the proper management of the freshwater resources (Rodell et. al., 2009).

The rise in human populations has coincided with increased pollution of freshwater (Ravera, 1978). Most water pollutants are anthropological in origin. Based on the source of pollutants, water pollution can be categorized as "Point Source Pollution", when the contaminants come from a single identifiable source, and "Nonpoint Source Pollution", when the pollution is a cumulative effect caused by multiple sources.

Major sources of these pollutants are industrial, agricultural and domestic in nature (Figure 2). The key pollutants are: untreated sewage, toxic and heavy metals (such as lead, cadmium, mercury, arsenic, etc.), plant pesticides and nutrients from agricultural runoffs (such as nitrates, phosphates, etc.), petrochemicals, radioactive agents (such as tritium, iodine, radon, cesium, uranium, etc.), inorganic chemicals form industrial and domestic sources (such as acids, salts, household cleaners, detergents, etc.), thermal water (water pumped from rivers or lakes and used as coolant for power plants and then discharging back into the source), among others. Polluted water is not only hazardous to human health, but also poses threat to aquatic lives.

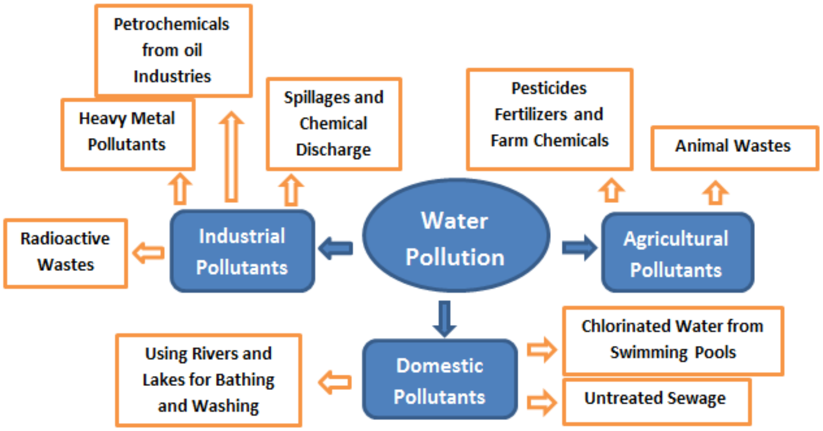
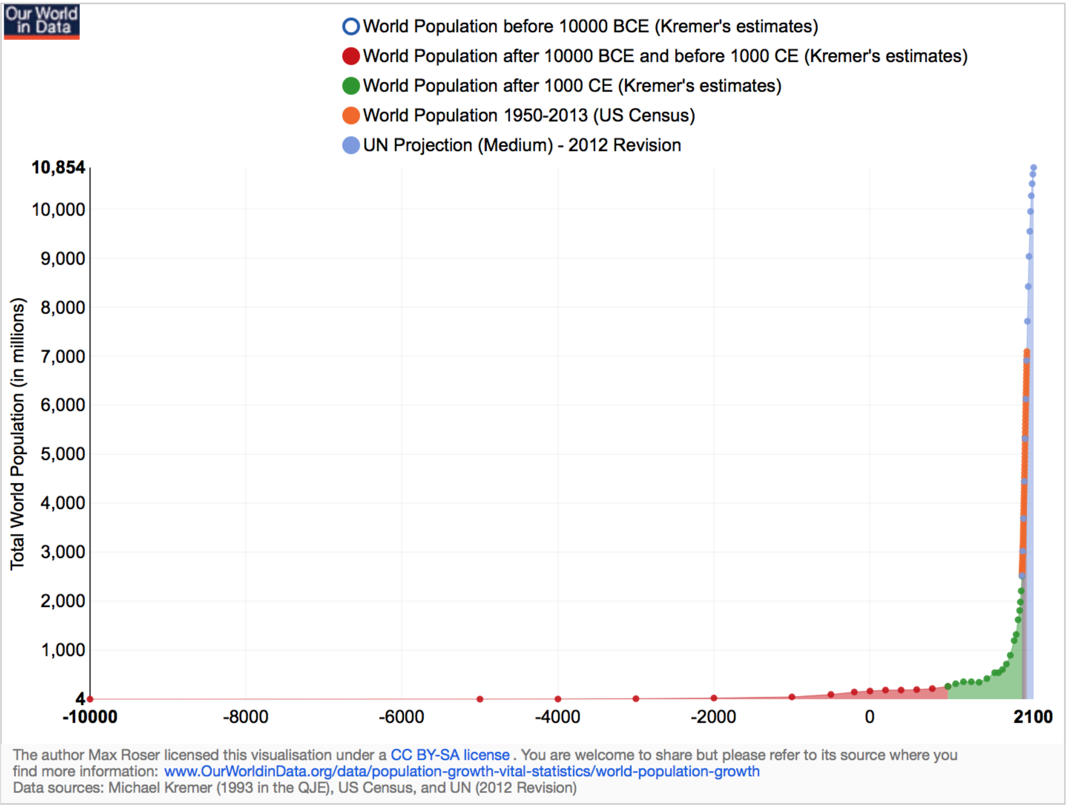


Figure 2. The above figure presents the major sources of freshwater pollution.

PREVENTION

Considering the sources of pollutants as discussed above, measures to prevent water pollution should be mostly common sense approaches. With the rapid growth in human population and expansion of industrial and agricultural activities, reducing pollutants that enter the water bodies, will be a daunting task. However, developing more efficient technologies, reducing domestic and industrial wastage, reducing consumption and recycling will definitely help to check the pollution status.

The annual rate of population growth has shown an inclination of decline. In 1962, population growth peaked at approximately 2.1% and has been cut in half since (Ortiz-Ospina and Roser, 2016). Thus, with education and birth control it is hoped that reproduction will decline to manageable levels. Otherwise, it could lead to turmoil and civil unrest with billions of people suffering the consequences. Despite this, others have projected a continued increase (Oriz-Ospina and Roser, 2016) an overcapacity reached by year 2100 (Figure 3).


World population over the last million years and projections 2010-2100[6]

The author Max Roser licensed this visualisation under a CC BY-SA license. You are welcome to share but please refer to its source where you find more information: www.OurWorldinData.org/data/population-growth-vital-statistics/world-population-growth
Data sources: Michael Kremer (1993 in the QJE), US Census, and UN (2012 Revision)

Figure 3. The above chart exhibits long-run historical perspective of world population growth. Data presented in this chart is collected from a number of sources and for different periods in history (Source: www.OurWorldinData.org/data/population-growth-vital-statistics/world-population-growth).

Additionally, depletion of forests to meet increasing land demands for agricultural and other human usage may also disrupt the surface water balance by disrupting natural water precipitation, evaporation, runoff, and groundwater flow. Surface runoff and river discharge generally increase when natural forests are cleared (Foley et al., 2005). Thus, unplanned deforestation has to be stopped to help the natural water cycle of the earth.

DISCUSSION AND CONCLUSION

Approximately 71% of the Earth's surface is covered by water with ocean's holding almost 97% of it (USGS, 2016b). This amounts to 326 million trillion gallons, which of course, is undrinkable (Gerbis, 2010). Although many have been able to convert seawater into drinking water, this has been cost prohibitive (Gleick, 2008). This type of conversion requires a great deal of energy and depending on the location and other factors, could cost over $2.00 to produce one cubic meter of fresh water from sea water (Ibid.). However, faced with worldwide shortages, this cost may become necessary if the expense cannot be reduced.

There is no question that freshwater is a valuable commodity on Earth. However, due to potentially warmer climates, pollution and overpopulation, it could become the most critical factor in the survival of life on our planet. In 1798 a British cleric and scholar named Thomas Malthus wrote *Essay on the Principle of Population* (Raven and Johnson, 2002) in which he showed that human growth was geometric but that food production was arithmetic. This led Charles Darwin and Alfred Russell Wallace to jointly propose the concept of evolution in which natural selection limits the extent to which any population could continue (Raven and Johnson, 2002). Thus, availability of water could have a direct effect on the evolution of every species on our planet.

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Impact Of Race, Poverty, Insurance Coverage And Resource Availability On Breast Cancer Across Geographic Regions Of Mississippi

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ABSTRACT

Introduction: The incidence of breast cancer was investigated in various health district, in the state of Mississippi. We have compared the Southwest region, public health district VII to the Delta region, public health district III, in regards to health care disparities and accessibility. These two regions differ significantly from the remainding 7 districts in breast cancer incidence, mortality rates, SEER (surveillance, epidemiology, end results) staging, insurance coverage and socioeconomic status. **Materials:** Data were collected from the Mississippi Cancer Registry for the years 2003-2013. The study was approved by University of Mississippi Medical Center Institutional Review Board (IRB) and was granted an exemption for de-identified data. A total of 20,083 records were obtained and a total of 20,008 were used in analysis. Data was segregated into individual groups based on health districts as defined by the Mississippi Department of Health. Two different statistical programs were used for analysis: STATA 14 and Excel 2016 statistical package. **Results:** In a global analysis, the nine public health districts were compared to each other. Then a comparison was made between the District VII and the remaining 8 other health districts, followed by specific comparison between District VII and District III. The incidence and mortality rates for breast cancer among African American women in Districts VII and III are statistically higher than in the other seven health districts of Mississippi. Further, African American (AA) women were diagnosed at an older age and later stage of disease based on SEER staging as compared to Caucasian (CAU) women. In addition, the majority of AA women were living in poverty, less educated, and had public health care providers. **Conclusions:** Public health District III is in the heart of the Mississippi Delta and has long been known for dramatic health disparities. This study indicated that the southwestern region of Mississippi, District VII is not statistically different from the traditional Delta. Future plans to improve the health of all Mississippians must now also include efforts to address health disparities in District VII as well as the Delta in order to achieve the Healthy People 2020 goals.

Keywords:

INTRODUCTION

Breast cancer is the most common type of cancer in women in Mississippi. It has been shown that African American women in Mississippi have an age specific incidence in the 40-49 years higher than Caucasian women (329.73 vs 239.08 per 100,000) with a corresponding age specific mortality rate of 89.1 per 100,000 for African American women compared to 28.66 per 100,000 for Caucasian women (Mississippi Cancer Registry, 2012). Although breast cancer rates have declined nationally since 1990 (DeSantis et al., 2016), this improvement has not been distributed across all segments of the population. Disparities have been associated with race/ethnicity (DeSantis et al., 2016; Jacobellis and Cutter, 2002; Weir et al., 2003), geographic status (Liff et al., 1991; Higginbothan et al., 2001; Coughlin et al., 2002; McLafferty and Wang, 2009; Markossian et al., 2014), and socioeconomic status (Bradley et al., 2002; Barry and Breen, 2005; Nichols et al., 2014). Other factors that have been proposed to account for these disparities in breast cancer outcomes include more advanced stage at diagnosis, fewer physician recommendations for mammography, underutilization of cancer screening, higher prevalence of obesity, poorer patient physician relationship, and higher rates of hypertension among ethnic minorities, as well as differences in insurance coverage (Coleman and O'Sullivan, 2001; Harris et al., 2003; Li et al., 2003; Maloney et al., 2006; Siminoff et al., 2006; Braithwaite et al., 2009; Sail et al., 2012; Robbins et al., 2014).

The Mississippi River Delta region consists of 252 counties or parishes in eight states near the lower half of the Mississippi River. Disease burden and mortality rates from all causes, including cancer and heart disease, in these delta counties are 10% higher than other non-Delta counties in the same states and 20% higher than rates for the rest of the United States as a whole (Felix and Stewart, 2005; Cosby and Bowser, 2008). At a state level, the Delta is considered to be primarily Health District I and III. District V, which includes Hinds, Madison, and Rankin counties, is arguably the largest metropolitan area of the state and has less in common with the traditional concept of the Delta. District VII in the southwest corner of the state is therefore physically separated from the Delta and is not typically thought of when discussions of health disparities associated with Districts I and III. In an effort to better understand the different problems facing each of the health districts in Mississippi, this project sought to address questions of breast cancer rates in the state.

Keeton (2014) noted that geography had a significant impact on the stage of breast cancer at which the patient was diagnosed. Mayfield-Johnson et al. (2016) reported that in Mississippi, the relative burden of invasive breast cancer varies by age and by race/ethnicity. Although these studies were quite comprehensive and compared some data for each health district as well as surrounding states, questions remain. In particular, we wanted to focus on District VII and compare it to the remainder of the state.

While great progress has been made in research on the elimination of health disparities in the past few years, further work is necessary in translating research to practice (Scarinci, 2009). Community-Based Participatory Research is a promising methodology that not only fosters research and capacity building, but also promotes ownership and sustainability by mobilizing underserved communities as political and social actors in the elimination of cancer disparities. The World Health Organization (WHO) defines health promotion as the "process of enabling people and communities to take control over their health and its determinants". Thus, by definition health should be promoted through community involvement in which community members decide what, when, where, and how health will be promoted and disease will be prevented in their communities (Scarinci, 2009).

The ultimate goal of health disparity studies is to reduce breast, cervical, and colorectal cancer disparities between African-Americans and Caucasians in underserved counties in Mississippi. Research focused on developing and implementing a community action plan that should lead to reduced health disparities between African-Americans and Caucasians in these counties in Mississippi. These goals can only be met with careful analysis of the differences between communities of need.

METHODS

Research Data Sources. The Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute (NCI) is an authoritative source of information on cancer incidence and survival in the United States. The SEER program has been collecting clinical, pathological, and demographic information on cancer patients since 1973. Data are available for Caucasians, African Americans, and all races combined since 1973 and for American Indian/Alaska Natives (AI/ANs), Asian/Pacific Islanders (APIs), and Hispanics since 1992. SEER incidence rates were adjusted for reporting delay.

Data Acquisition and Analysis. Cancer Registries collect and publish data for cancer researchers, public health officials, academic centers and the public to use. We obtained the records for breast cancer patients in Mississippi from 2003-2012. Data included race, age at diagnosis, county, SEER stage at diagnosis, and primary payer at diagnosis. A total of 20,083 records were reviewed and ultimately 67 records were excluded for either race other than African American or Caucasian (excluded Hispanics and Asians due to very small number), and military as primary payer.

Variables requested included date of diagnosis, primary site, histology, age at diagnosis, race, sex, primary payer at diagnosis, stage of disease at diagnosis, county, and census tract. The study population included females diagnosed with breast cancer for the years 2000-2012. The data did not include those cases of Mississippi residents obtained from other state cancer registries, the Veteran Administration hospital system or the Keesler Air Force Base Hospital. We followed protocols previously described (Adams et al., 2009). Data from the MRC does not provide any private health information which would be subject to HIPPA regulations. This project (IRB File #2014-0244) was submitted to the UMMC Institutional Review Board and received an exemption.

Statistical Analysis. For the analysis of different regions of Mississippi, we relied on the Mississippi Department of Health designations. We used the STATA computer statistical package, Version 14. To assess the association among patient characteristics, area-level attributes, clinical characteristics, and breast cancer-related outcomes, we compared frequencies and measures of central tendency using the chi-squared test of significance and the t-test. A one sample t-test allowed us to test whether a sample mean (from a normally distributed interval variable) significantly differs from a hypothesized value. For example, if the two groups are included in the independent variable of race with two levels, Caucasian and African American, it is appropriate to use a t-test to determine whether they differ. For comparison of groups, it is also important to include the Pearson Chi Square test and a linear model using one way ANOVA. The dataset comprises 12 data fields divided into sections dealing with demographic data and pathology descriptions.

Socioeconomic Status. The percentage of the population with a household income below the federal poverty level (FPL) is the most important indicator of area-level SES and correlates well with other SES measures (Singh et al. 2004; Krieger 2005). Following the Office of Management and Budget's (OMB) Statistical Policy Directive 14, the Census Bureau uses a set of money income thresholds that vary by family size and composition to determine who is in poverty. To characterize county-level SES, we obtained that percentage from the US Census 2010. Data for each health district was compared using standard statistical algorithms in Excel 2016.

RESULTS

Mississippi Breast Cancer Rates

In the United States, the rate of getting breast cancer (incidence) varies from state to state. In Mississippi, we have among the lowest rates of breast cancer incidence nationwide (106.3-114 per 100,000 population). Overall, Mississippi ranks in the lowest quartile for female breast cancer rates. In neighboring Louisiana, the rate is 118.7-125.0 per 100,000, in the second highest quartile nationally while Tennessee and Alabama rank in the third quartile at 114.1-118.6 per 100,000. Arkansas did not meet the criteria for reporting. Unfortunately, compared to the rest of the United States, Mississippi did not fare as well when the mortality rates for female breast cancer were compared. In spite of having among the lowest incidence rates for breast cancer, we in Mississippi are in the top tier for mortality at 23.4-30.4 deaths/100,000, the top quartile in the nation. Our Deep South neighbors Louisiana, Alabama, and Georgia share the same high mortality rates. This is well above the target of 21 deaths per 100,000 set by the National Institutes of Health and the CDC in their Health People 2020 Caucasian paper (https://www.healthypeople.gov/2020/topics-objectives/national-snapshot/female-breast-cancer-deaths-2001%E2%80%932011).

Mississippi Cancer Registry Data

Data was obtained for all female breast cancer diagnosis in Mississippi for the years 2003-2012. Analysis of breast cancer records obtained from the MCR was done in three parts. First, the differences between all nine Mississippi Health Districts were analyzed to determine whether there were statistically significant differences for each of the categories. Second, District VII was compared to all other health districts. Finally, District VII was compared to District III, which comprises the major portion of the Delta region.

Analysis of All Nine Health Districts

In Mississippi, the population at risk for the development of breast cancer varies by health district. Most of Mississippi is comprised of rural areas, with major urban areas found in District V (Jackson metropolitan area), District II (DeSoto County and Southaven area), District VIII (Hattiesburg area), and District IX (Gulf Coast area). As shown in Figure1 (Panel A), there are differences in the incidence of breast cancer in women between the health districts. The highest rates are in Districts I and V (134.74-155.36 per 100,000), followed by Districts VIII and IX (133.19-133.63 per 100,000), Districts II and VII (129.62-131.76 per 100,000), and Districts III, IV, and VI (119.62-129.29 per 100,000). As seen in Figure 2 (Panel A), the highest incidence rates in Caucasian women are found in Districts I and V (135.11-152.69 per 100,000), Districts IV and IX (131.12-134.59 per 100,000), Districts I and VIII (129.77-130.99 per 100,000), and followed by Districts III, VI and VII in the lowest quartile. In contrast, the incidence rates for African American women (Figure 2, Panel B) are highest in Districts V and VIII (141.47-157.85 per 100,000), Districts I and VII (135.22-140.47 per 100,000), Districts I and IX (131.61-134.31 per 100,000), and Districts III, IV, and VI (125.52-130.92 per 100,000). These numbers are in sharp contrast to national trends in which Caucasian women have a higher incidence of breast cancer than African American women. In Mississippi, this is reversed: African American female Mississippians have a higher incidence of breast cancer than Caucasian female Mississippians.

Panel A: Incidence Rates, all races. **Panel B: Mortality Rates, all races.**

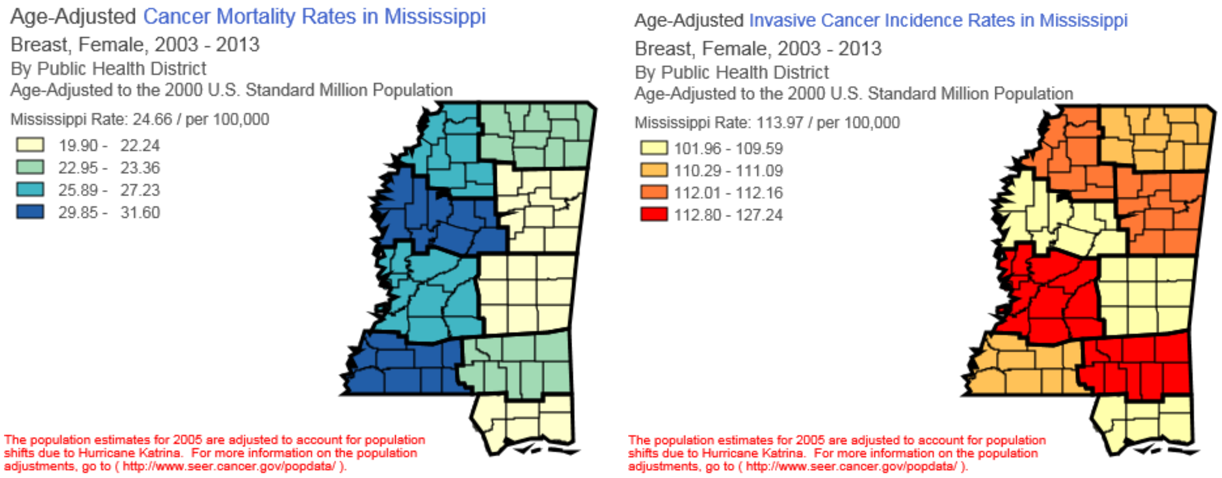


Figure 1. Age Adjusted Cancer Incidence and Mortality Rates in Mississippi by Health District. Included all female breast cancer, 2003-2013. All rates per 100,000.

Panel A. **Panel B.**

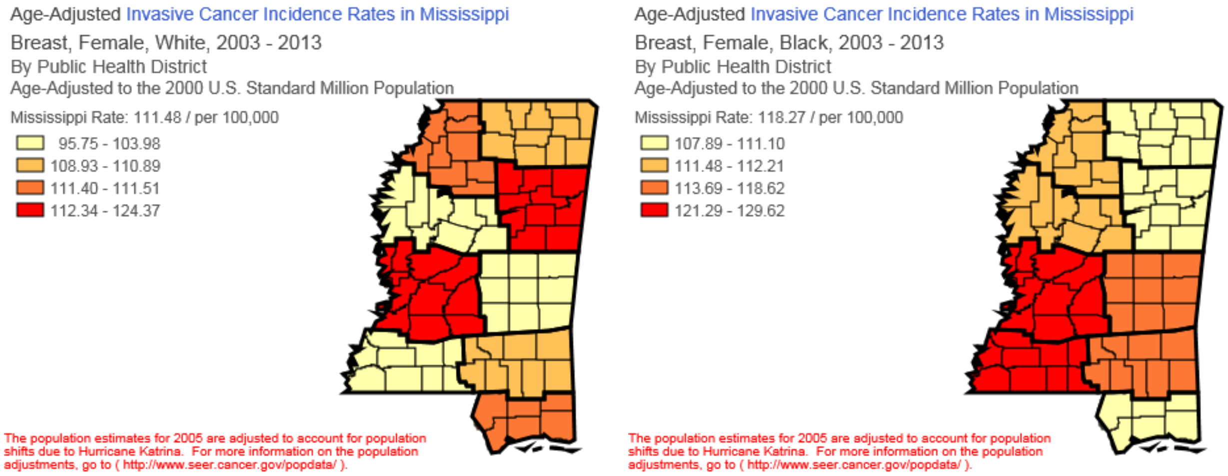
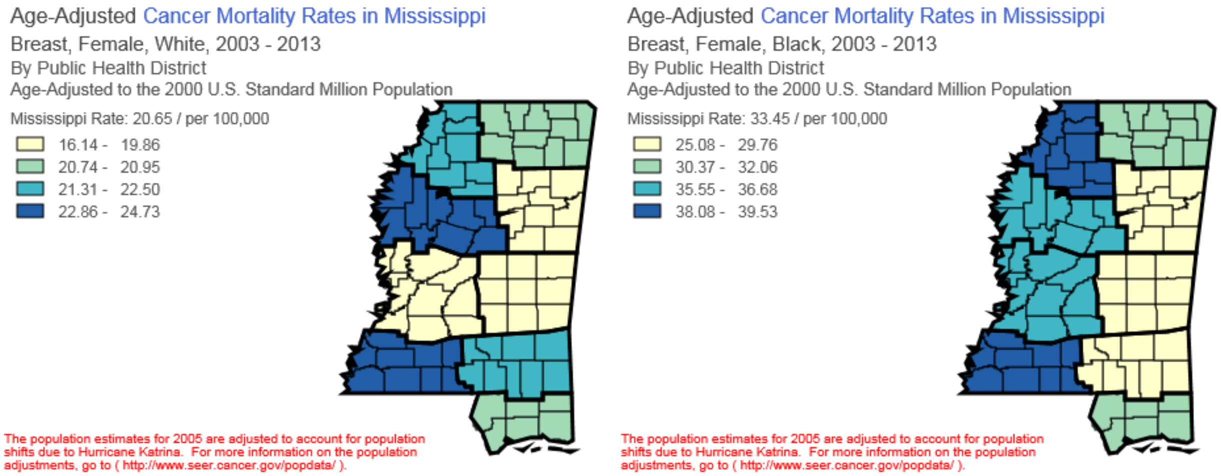


Figure 2. Age Adjusted Breast Cancer Incidence Rates In Mississippi by Health District. Included all female breast cancer, 2003-2013. All rates per 100,000. Panel A, Caucasian; Panel B, African American.

When mortality rates for breast cancer were compared by health district, racial differences became even more pronounced. Overall, the highest mortality rates are in District III and VI (29.85-31.60 per 100,000) (Figure 1, Panel B), districts which ranked in the lowest and highest quartiles for incidence of breast cancer, respectively. These two districts also have the highest mortality rates among Caucasian women (Figure 3, Panel A), at 22.86-24.73 per 100,000. In contrast, Districts I and VII have the highest mortality rates among African American women at 38.08-39.53 per 100,000 (Figure 3, Panel B). Thus, in the Delta, District III, the mortality rate for African American women is in a lower quartile than the rate for incidence of breast cancer in African American women. In District VII, the incidence rate among African American women is in a lower quartile than mortality rates. This may suggest more interventions or less severe disease in District III (Delta) than District VII. It is also important to note that the rates for African American women are higher than those for Caucasian women, corresponding to national trends in mortality rates.



Panel A. Caucasian Women

Panel B: African American Women.

The population estimates for 2005 are adjusted to account for population shifts due to Hurricane Katrina. For more information on the population adjustments, go to (http://www.seer.cancer.gov/popdata/).

The population estimates for 2005 are adjusted to account for population shifts due to Hurricane Katrina. For more information on the population adjustments, go to (http://www.seer.cancer.gov/popdata/).

Figure 3. Age Adjusted Breast Cancer Mortality Rates In Mississippi by Health District. Included all breast cancer in women, 2003-2013. All rates per 100,000.

Race. Analysis of breast cancer rates by race for all of the nine health districts revealed that the incidence of breast cancer was greater in Caucasian women in eight of the nine health districts, conforming to national statistics (Figure 1). Only in District III did the number of African American women diagnosed with breast cancer exceed that of Caucasian women (Figure 4). Thus, the outlier in this analysis is District III, accounting for the statistical difference detected when all nine health districts were compared (Table 1; Pearson chi2 = 1.4e+03, and the p value = 0.0001; statistically significant).

Age Groups. For analysis, breast cancer patients were stratified into five age groups: 18-34, 35-44, 45-54, 55-64, and 65-89 years of age (Figure 5). Data was not collected from patients greater than 89 years of age since that might allow identification of individuals in some of the smaller, less populated counties and districts, as noted by the Institutional Review Board. All districts had greater number of patients diagnosed at an older age, following national trends. Breast cancer tends to occur more frequently in women over 60. In addition, the largest number of patients in any age group is in District V (Jackson to Vicksburg corridor), although women in this district tend to be diagnosed earlier. This is the source of the statistical difference when looking at all nine districts. The total number of patients diagnosed in every age group was greatest in District V, which includes the largest urban area of the state. However, the percentage of breast cancer diagnoses in District V is higher only in age groups 35-44 and 45-54 while it decreases relative to seven other districts in age group 55-64 and all other districts in the highest age group. Therefore, District V is the outlier in this statistical analysis (Table 1; Pearson chi2 = 101.7624; p = 0.0001).

SEER Stage at Diagnosis. Breast cancer is diagnosed based on location of the tumor as being local, regional or distant from the breast tissue. More advanced cancers spread from local to regional and finally distant sites. This progression is associated with poor prognosis, more advanced disease, and delayed diagnosis. When the districts were compared for SEER diagnosis of breast cancer, statistical analysis found significant differences (Figure 6; Table 1; Pearson chi2 = 99.8213; p = 0.0001).

Districts I, III, and VII have the highest percentage of patients with diagnosis at distant sites, indicative of more advanced disease and a poorer prognosis. In many studies, parts of District I are included with District III to define the Mississippi Delta. District VII in the southwest of the state is comparable. These districts statistically differ from the rest of the state. This also correlates with maps of breast cancer mortality rates in Mississippi. One possible explanation for more advanced disease may be delayed diagnosis.

Primary Payer. Barriers of care frequently include lack of insurance as a contributing factor to increased mortality due to breast cancer. We examined the rates for private payer, Medicare and Medicaid as primary payer by health district (Figure 7). Overall, less than 20% of breast cancer patients had unknown/none listed for insurance. Surprisingly, more individuals in Mississippi reported having private insurance than those on public insurance. However, there were statistical differences in these rates among the health districts. District V had the lowest rate of private insurance and the highest rate of Medicaid, accounting for the statistical difference noted when comparing all nine health districts. District VII does not appear to be statistically different in and of itself. There were a substantial number of records for which no payer was identified. Statistical analysis revealed significant differences between the districts (Pearson chi2 = 387.0682; p = 0.0001).

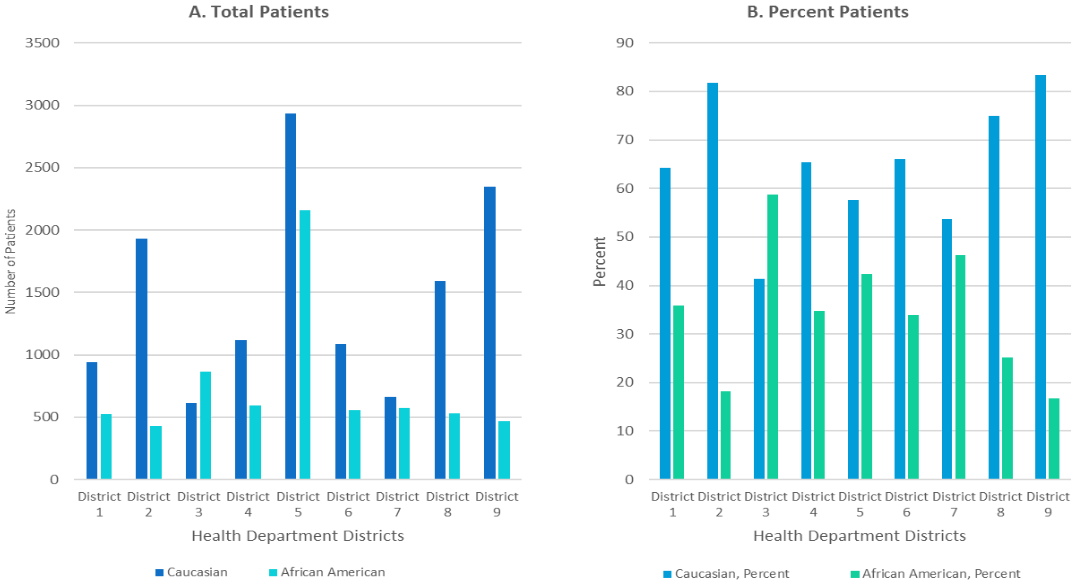


Figure 4. Comparison of Breast Cancer Rates by Race, All Districts. Panel A, total patients; Panel B, percent patients.

*Note that in this and subsequent figures, health districts are referred to by Arabic numerals rather than the Roman numeral system employed by the Department of Health. This allowed the data to be presented in order from left to right. When Roman numerals were employed in the Excel program used to generate the graphs, District IX (9) appeared in the center of the graph between District IV and V.

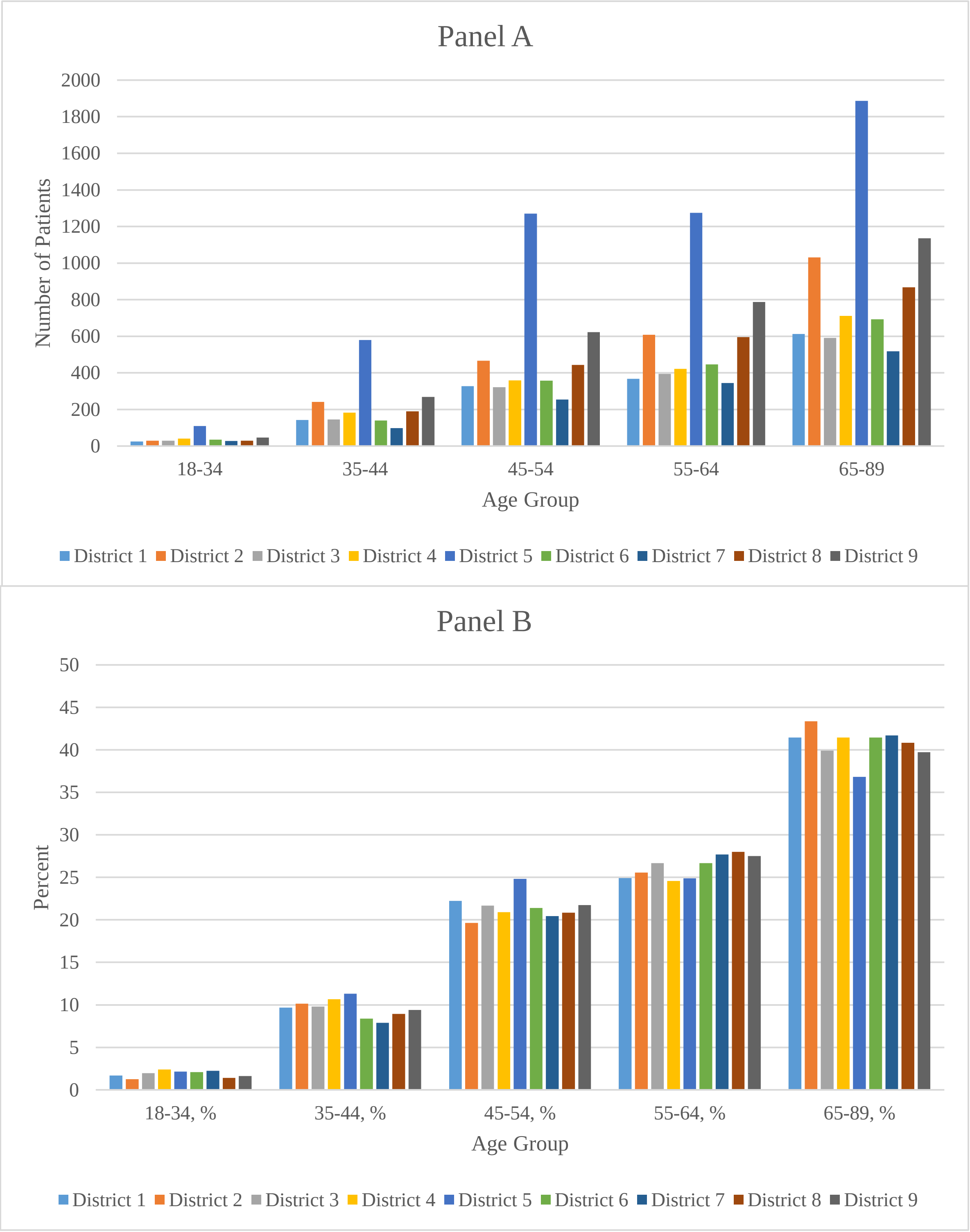


Figure 5. Comparison of Breast Cancer Patients by Age Group and Health District Panel A, total patients; Panel B, percent patients.

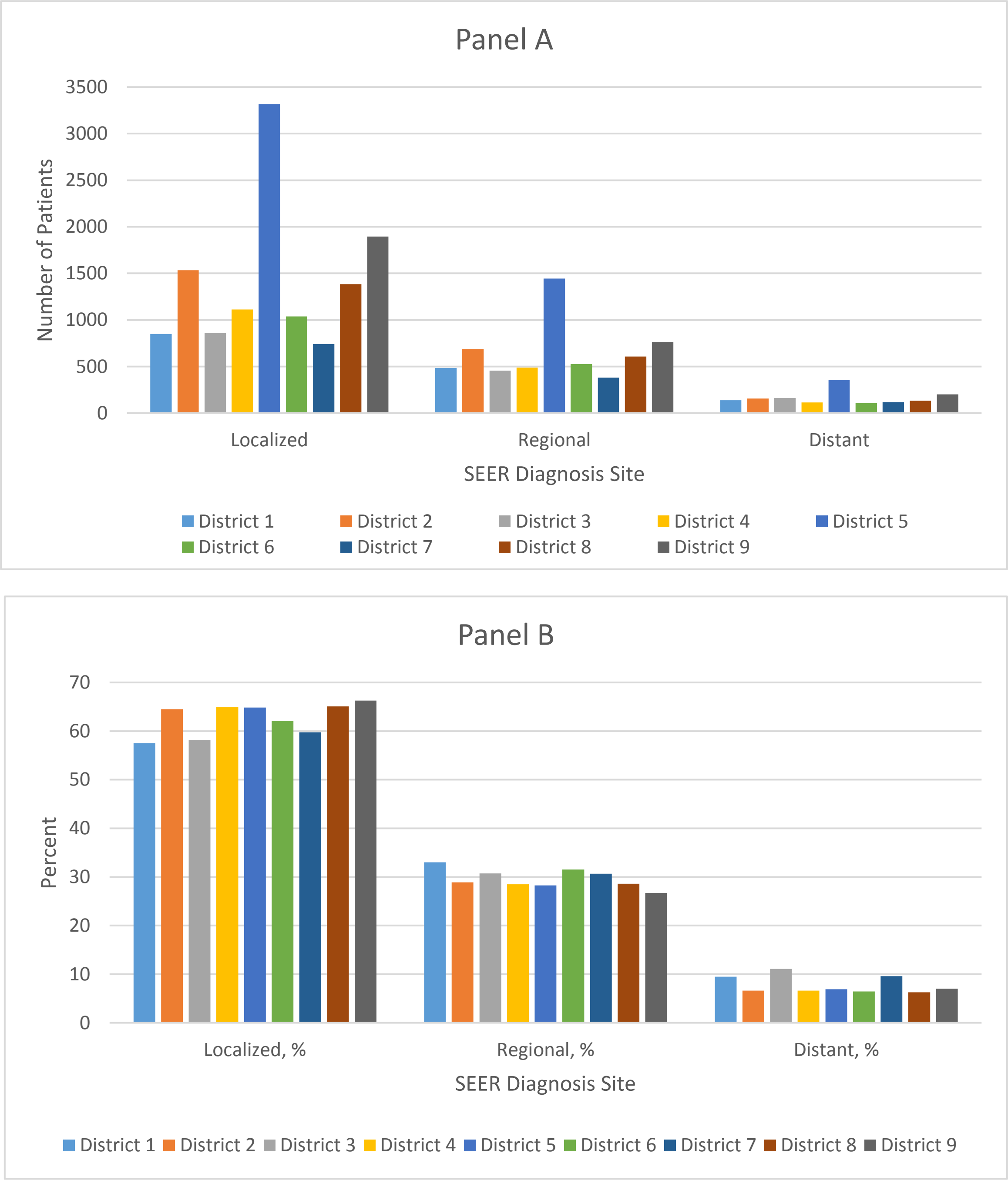


Figure 6. Comparison of Breast Cancer SEER Diagnosis by Health District. Breast cancer was categorized as localized, regional or distant as described in the NCI SEER Manual. Panel A, total patients, Panel B, percent patients.

Primary Payer. Barriers of care frequently include lack of insurance as a contributing factor to increased mortality due to breast cancer. We examined the rates for private payer, Medicare and Medicaid as primary payer by health district (Figure 7). Overall, less than 20% of breast cancer patients had unknown/none listed for insurance. Surprisingly, more individuals in Mississippi reported having private insurance than those on public insurance. However, there were statistical differences in these rates among the health districts. District V had the lowest rate of private insurance and the highest rate of Medicaid, accounting for the statistical difference noted when comparing all nine health districts. District VII does not appear to be statistically different in and of itself. There were a substantial number of records for which no payer was identified. Statistical analysis revealed significant differences between the districts (Pearson chi2 = 387.0682; p = 0.0001).

District VII versus All Other Districts

As noted above, differences exist between the health districts in regard to breast cancer parameters. District VII is one of the districts that has striking differences. It is statistically different from the other eight combined districts in every category.

Race. District VII was compared to the other eight districts combined. District VII has a statistically higher proportion of African American women with breast cancer than the combined other eight health districts (Figure 8, Panels A and B; Table 1; Pearson chi2 = 94.7167; p = 0.0001). However, District VII does follow national trends in that there are more Caucasian women diagnosed with breast cancer than African American women. Note that we excluded Hispanics due to small numbers. The p-value indicates that the distribution of African American and Caucasian women is different between District VII and all the other districts combined. This is demonstrated by the racial makeup of District VII where African Americans make up 46.29% of breast cancer patients when compared to 32.81% in all other districts (combined).

Age Group. When District VII and all other districts combined were compared based on age group, again District VII differs significantly from the remainder of the state (Figure 8, Panels C and D; Table 1; Pearson chi2 = 10.1717; p = 0.038). However, the difference was not as marked as for other comparators. Further, the differences were skewed in District VII: percentage of patients was lower for the 34-44 and 45-54 age groups but higher for the remaining age groups of 55-64 and 65 and over than the other districts combined. A trend toward higher age at diagnosis may signal a more advanced disease. This may contribute to the higher mortality in District VII noted above. Again, this later diagnosis correlates to poorer outcome noted nationwide.

SEER Stage at Diagnosis. SEER stage at diagnosis is an important indicator of morbidity and mortality. District VII had relatively more patients diagnosed with regional and distant tumors than the rest of the state (Figure 8, Panels E and F; Table 1; Pearson chi2 = 12.1; p = 0.002). It is tempting to speculate that the more widely disseminated the tumor (regional or distant as opposed to local) at diagnosis may reflect delayed diagnosis as noted for age at diagnosis (increased in District VII in the two highest age groups) described above.

Primary Payer. When District VII was compared to the remainder of the state for primary payer, there was a statistically significant difference (Figure 8, Panels G and H; Table 1). District VII has statistically more patients reporting private payer and fewer Medicaid payer at time of diagnosis than the other eight health districts. More District VII patients listed private insurers (54.43%) as the primary payer at time of diagnosis compared to the remainder of the state (50.77%). District VII also had slightly more women reporting Medicare as a source of insurance compared to the rest of the state, perhaps reflecting the higher age at diagnosis. These differences were statistically significant (Pearson chi2 =17.91; p = 0.0001).

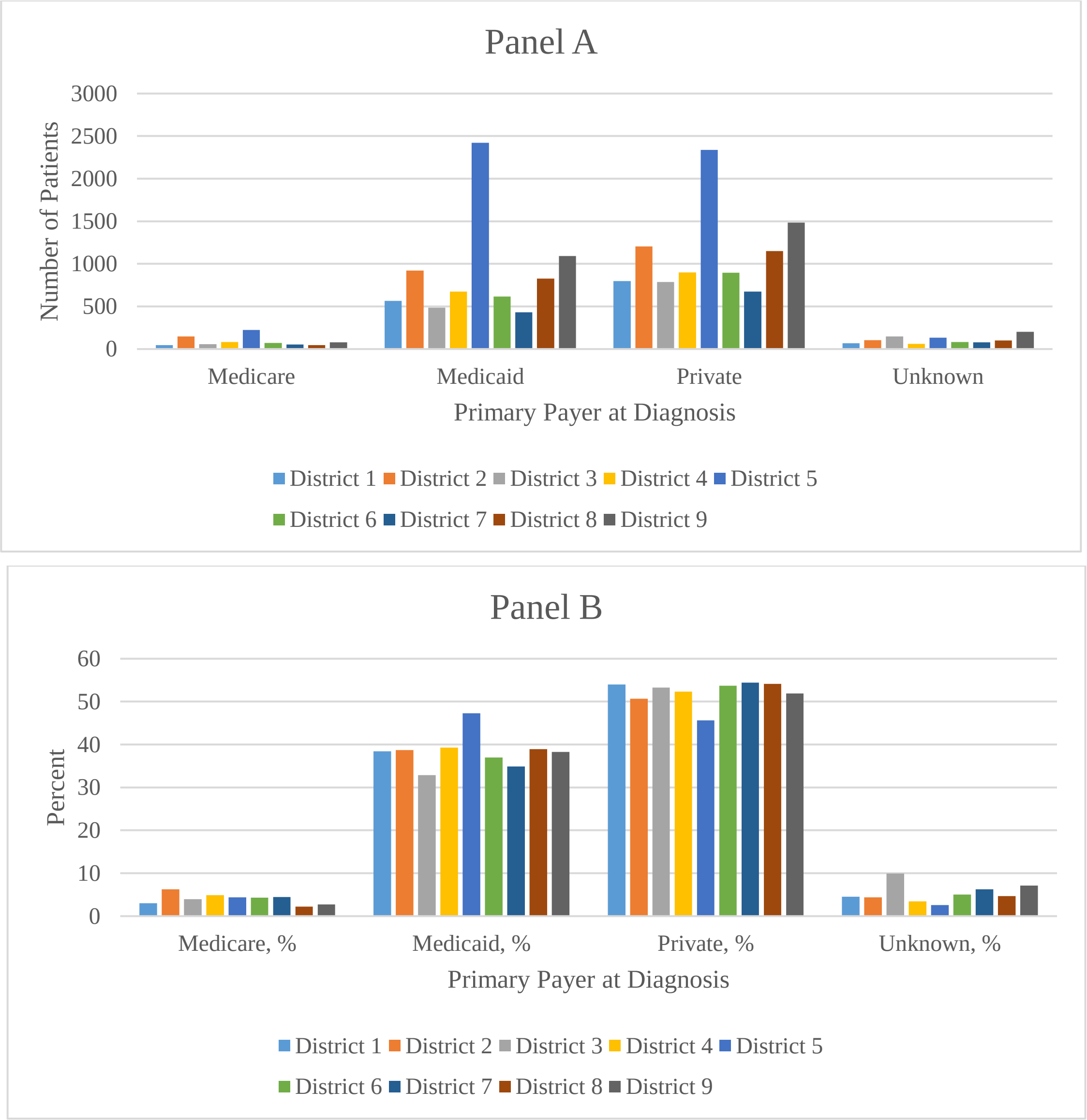


Figure 7. Distribution of Payer Type at Diagnosis by Health Districts. Categories included Medicare, Medicaid, and Private payers. Military payers were excluded. Panel A, total patients; Panel B, percent patients

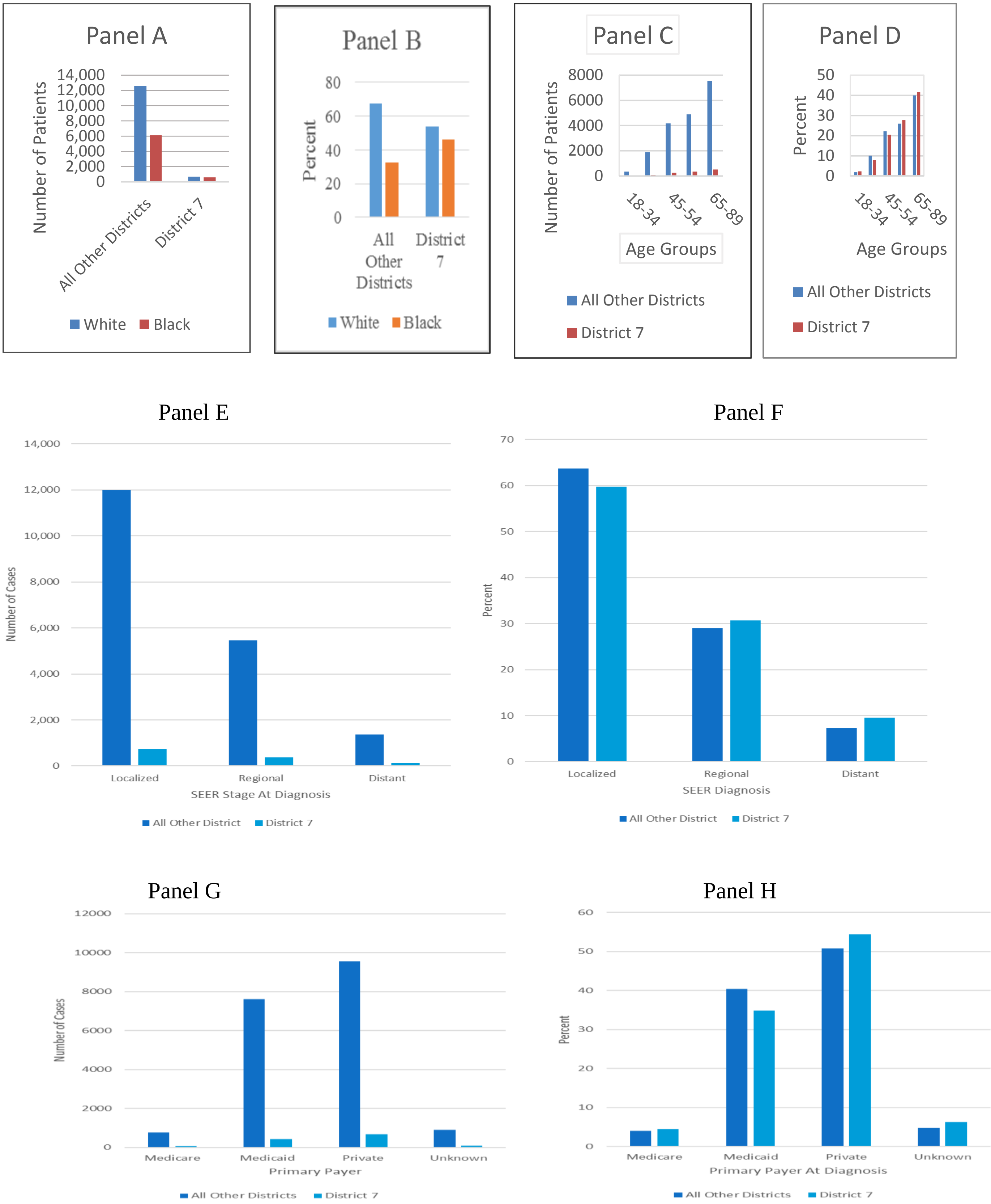


Figure 8. Comparison of District VII t o all oOther Health Districts. Panels A and B, race; Panels C and D, age group; Panels E and F, stage at diagnosis; and Panels G and H, primary payer.

Table 1. Statistical Comparisons: All Districts, District VII vs All Others, District VII vs District III.

Summary of Statistical Analysis: Pearson χ^2 and P values.

	All Districts		District VII vs All Others		District VII vs Delta	
	Pearson χ^2	p value	Pearson χ^2	p value	Pearson χ^2	p value
Race	1.4 X 10^3	<0.0001	94	<0.0001	0.335	0.563
Age Group	101.8	<0.0001	10.1	0.038	6.4	0.171
SEER Diagnosis	99.8	<0.0001	12.1	0.002	1.34	0.512
Primary Payer	387.1	<0.0001	17.9	<0.0001	3.48	0.323

Table 2. Comparison of District VII and District III: Education Levels.

	District VII		District III		P Value	Pearson Correlation
Less than High School	21.2%	±3.4%	27.0%	±3.8%	0.008	-0.27
High School Degree	35.7%	±2.4%	30.6	±3.6%	0.0007	0.47
Some College	27.8%	±3.7%	26.8%	±2.3%	0.28	-0.49
College Degree or Greater	14.6%	±1.9%	15.6%	±3.3%	0.15	-0.107

District VII versus Mississippi Delta (District III)

Race. The next analysis compared District VII to District III, the major portion of the Mississippi Delta (Table 1). Although some analyses have included District I and District III as the Delta, for this analysis only District III was employed to represent the Delta. As discussed above, in District III the number of African American women diagnosed with breast cancer exceeded that of Caucasian women, serving as the outlier in the analysis when all nine health districts were compared. The racial composition of breast cancer patients in the Districts III and VII are not significantly different (Pearson chi2 = 0.3352; p = 0.563). Thus, these two districts are quite similar.

Age Group. Age at diagnosis was not significantly different between District VII and the Delta (District III). As noted above, District VII does differ from the remainder of the state but not from District III specifically (Table 1). This later age at diagnosis in Districts III and VII may contribute to poorer prognosis as discussed previously. However, it is important to recall that District VII has the highest quartile rating for mortality due to breast cancer while District III has a lower mortality. The percent of patients diagnosed is slightly higher in District VII compared to District III in the 55-64 (27.7% and 25.8%, respectively) and 65-89 (41.1% and 40.68%) age groups (Pearson chi2 = 6.4; p = 0.171).

SEER Stage at Diagnosis. Another important indicator of poor prognosis is SEER stage at diagnosis. District VII was found to be statistically more likely to have more advanced stage of disease when compared to the other eight districts (Table 1). When the SEER stage at diagnosis was compared for District VII and the Delta (District III), the two districts do not differ significantly in the distribution of disease at local, regional, or distant sites (Pearson chi2 = 1.34; p = 0.512). Taken together with age at diagnosis, this finding would imply a poorer prognosis and increased mortality in both of these districts. Indeed, the mortality rates for the two districts are the same overall and for Caucasian women. However, when mortality rates for African American women are examined, African American women in District III were lower than those in District VII.

Primary Payer. Primary payer status was also evaluated for Districts III and VII. These are the two smallest health districts in terms of the at risk population. As noted, District VII had statistically more patients reporting private payer and fewer Medicaid payer at time of diagnosis than the other eight health districts. District VII and the Delta (District III) were compared for the primary payer at diagnosis. There was no significant difference between the two (Table 1; Pearson chi2 = 3.48; p = 0.323). Less than 10% of breast cancer patients in these two districts were reported as unknown/no coverage. Therefore, lack of insurance is less likely to be a contributor to disparities in breast cancer care in these two rural districts.

Socioeconomic Status. Several reports have indicated the importance of socioeconomic status in health disparities. Therefore, data from the US Census Bureau was used to compare Districts VII and III. Three major indicators were considered: education levels (less than high school, high school degree, some college, and college degrees), percent of the population living in poverty (national poverty level), and median household income. When determining socioeconomic status, education is a major component. As shown in Table 2, we determined the percentage of individual in District VII and District III with less than high school, high school degree, some college, and a college degree from census data. Lower educational levels equate with lower socioeconomic status. District III has statistically more individuals with less than a high school degree. Both districts have equivalent populations with some college or a college degree. Therefore, District III would rank lower in this aspect of socioeconomic standards.

Another parameter considered in the assessment of socioeconomic status was median household income. The percentage in poverty between District VII and III were 27.6 ±6.27 and 32.72±8.79. Statistically, the two districts are not different, p=0.079, Pearson correlation=0.171. Further, the median house incomes in the two districts are not statistically different: District VII $31,299 ±3,268 and District III $29,573 ± 4,525 (p= 0.117, Pearson correlation = 0.32).

DISCUSSION

Breast cancer is the most common cancer among women in Mississippi with nearly 2500 new cases diagnosed each year. Racial disparities in breast cancer outcomes have been well documented with African American women having a lower incidence of breast cancer compared with Caucasians but a higher overall mortality. In Mississippi, the relative burden of invasive breast cancer varies by age and by race/ethnicity. In 2012. The age-specific incidence for breast cancer among African American women aged 40 – 49 years was 329.73 per 100,000; whereas, the same rate among Caucasian women was 239.08 per 100,000. However, the age-specific mortality rate for African American women was 89.10 per 100,000 and was 28.66 per 100,000 for Caucasian women. Even after age 50 African American women continue to have the highest mortality rate (Mayfield-Johnson et al., 2016).

Health disparities between racial groups have been extensively researched and cancer statistics are reported each year for major racial and ethnic groups (Siegel et al., 2012; Howlander et al, 2012). African American women, especially in the Deep South and Mississippi in particular, present a paradox: although they tend to have lower incidence rates of breast cancer, they have higher mortality rates. Incidence rates in African American women compared to Caucasian women are higher District VII and our analysis indicts that this is statistically significant. This is in contrast to the national trends in incidence rates for breast cancer.

There were statistical differences between the nine health districts for all parameters compared. Race was significant for District III (Delta). In Delta, more African American women than Caucasian women were diagnosed with breast cancer, in contrast to national trends. Indeed, in Mississippi, African American women had higher incidence rates per 100,000 than Caucasian women in every district. When mortality was considered, Districts III and VII have the highest mortality rates, for African Americans and Caucasians combined.

Age of diagnosis was found to be most significant in District V, where diagnosis was made at an earlier age. District V includes the state capital and is the most densely populated region of the state. District V is host to the largest number of hospitals and highest concentration of physicians in the state (Board of Medical Licensure, MS, accessed Nov. 23,2016 at http://www.msbml.ms.gov/msbml/web.nsf/webpages/Stats_Stat2010?OpenDocument). The availability of these medical resources likely plays a major role in the early diagnosis. Multiple clinics and the presence of the University of Mississippi Medical Center provide care for large numbers of patients with Medicare/Medicare. Further, specific programs have been employed to improve women's knowledge of the risk factors for developing breast cancer, how to perform breast self-exams and promote mammography (Wilson-Anderson et al., 2013). This program, based in Vicksburg and targeting 2 rural counties in the Delta was a partnership between the University of Mississippi School of Nursing and the Sisters of Mercy. African American women were enrolled in educational classes and trained to become health advocates within their communities. Such grass roots projects may have played a role in the earlier intervention and diagnosis in District V.

Breast cancer is diagnosed based on location of the tumor as being local, regional or distant from the breast tissue. More advanced cancers spread from local to regional and finally distant sites. This progression is associated with poor prognosis, more advanced disease, and delayed diagnosis. Districts I, III and VII have the highest percentages of patients diagnosed at distant sites. District VII in the southwest of the state is comparable to Districts I and III for stage of disease at diagnosis. These districts statistically differ from the rest of the state.

Barriers of care frequently include lack of insurance as a contributing factor to increased mortality due to breast cancer. Patients with breast cancer were compared for their primary payer at diagnosis. Categories included private payer, Medicare, and Medicaid by health district. Overall, less than 10% of breast cancer patients had unknown/none listed as primary payer. Most patients statewide had private insurance. There were statistical differences in health districts. District V had the lowest rate of private insurance and the highest rate of Medicaid, accounting for the statistical difference noted when comparing all nine health districts. District III had the highest number of unknown/none recorded as payer at time of diagnosis. Thus, the highest rate of uninsured and highest rate of SEER site at diagnosis (distant) both occurred in District III.

When District VII was compared to all other districts, it was found to be statistically different in all categories. District VII has statistically more African American women diagnosed with breast cancer than the combined other eight health districts. Women in District VII are more likely to be diagnosed at an older age, outpacing all combined districts in both the 55-64 and 65-89 age group while they are less likely to be diagnosed in the 35-44 and 45-54-year-old age groups. Women in District VII are diagnosed with more advanced disease than the remainder of the state, with more regional and distant SEER stage at diagnosis. The combination of later age at diagnosis and advanced disease state correlate with the higher rates of mortality due to breast cancer in District VII, the highest quartile in the state and comparable to District I. Interestingly, compared to the combined other eight districts, District VII had a higher number of breast cancer patients with private insurance, fewer with Medicaid, and more with unknown/none listed as primary payer at diagnosis.

The Mississippi Delta is defined by the Delta Regional Authority as 252 counties or parishes in eight states near the lower part of the Mississippi River. In Mississippi, this area includes parts of public health districts I, III, V, and VII. However, examination of the Mississippi Cancer Registry public database, a request of a comparison of the Delta and the non-Delta regions of Mississippi is more limited and excludes District VII. For this study, we therefore compared the "heart" of the Mississippi Delta (District III) to the southwest region of the state, District

VII. The two districts are similar in overall demographics (size, rural area, similar geography). Indeed, in our analysis, the two districts did not differ in any of the parameters used to compare breast cancer patients: race, age at diagnosis, SEER stage at diagnosis, primary payer at diagnosis or socioeconomic status. This suggests that in considering distribution of resources and use of specific resources aimed at improving health outcomes, District VII and District III should be considered together and to have similar problems. The measures of public health and disease burden for Delta counties, including the southwest portion of the state, are higher in these regions than in the remainder of the state and the United States as a whole. Mortality rates from all causes, cancer, and heart disease are approximately 10% higher in this area of Mississippi than the rest of the state and 20% higher than the rest of the United States (Felix and Stewart, 2005; Cosby and Bowser, 2008).

Recently, Gennuso and colleagues (2016) conducted a study of key contributors to health outcomes and health disparities between Delta and non-Delta counties in eight states in the Mississippi River Delta Region. They found similarities between Delta and non-Delta in a number of health factors, including tobacco use, diet, and exercise, that predicted some poor outcomes related to self-related health. However, variation was most notable for predictors of mortality where the Delta fared worse than non-Delta regions. They concluded that the health of the populations living in the Delta counties is poorer than that for non-Delta counties within the same states because of a more general set of health factors that contribute to outcomes and that there are no unique set of health predictors in these poor outcome areas.

Socioeconomic status is often measured as a combination of education, income and occupation. It is commonly conceptualized as the social standing or class of an individual or group. When viewed through a social class lens, privilege, power, and control are emphasized. Furthermore, an examination of socioeconomic status as a gradient or continuous variable reveals inequities in access to and distribution of resources. The factors that are usually considered in establishing SES are income, occupation, education, neighborhood, and political power. The Mississippi Delta is one of the poorest regions in the nation. Districts VII and III certainly meet the criteria of poor, disadvantaged and frequently forgotten.

Limitations of the Study. Although a large body of data is available through SEER and MCR, more needs to be known. The records were incomplete for a number of factors that may play a role in breast cancer incidence and outcome. These include genetic markers, many of which are now available but do not date back to the early part of the data base accessed, as well as a host of risk factors associated with life-style. Rates of alcohol and tobacco use are not always reliable. Obesity is also important but not specifically collected for the SEER database. Other factors include the availability of local resources, including community support, transportation availability, and childcare for patients during healthcare visits. Cultural attitudes also play a role and are not easily summarized.

Health is a holistic term that includes biomedical, social, and psychosocial aspects. Heath has been shown to vary spatially: locally (different parts of towns or cities), regionally (Delta and non-Delta counties), nationally (Mississippi versus Colorado), and internationally (Japan fares much better than the United States on most measure of health) (Bambra, 2016). Thus, the place that an individual lives is composed of social, economic, and political relations as well as physical resources. The inequalities in health are therefore a result of a complex mix of economic, social, environment and political processes: places may be health promoting or health damaging (Bambra, 2016).

Studies such as that of Keeton (2014), Mayfield-Johnson (2016), and Dwyer- Lindgren (2016) support the approach employed here. State and county health departments should use county level data to identify local needs and develop policies and programs to fit very specific geographic regions. Physicians and researchers could focus more attention on unique local social, economic, and cultural differences that impact health disparities. Communities could more effectively advocate for change and education, to improve early diagnosis and appropriate treatment coupled to an improved support system.

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Differential Effects of Calcitriol, FGF-23, and Klotho on Vascular Smooth Muscle Cell Calcification and Their Role in Medial Calcification

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ABSTRACT

Background: Medial calcification is pathological mineral deposition in the middle layer of arteries. It is common in chronic kidney disease (CKD) patients and causes an increased risk for cardiovascular complications. Calcitriol, the active form of vitamin D, is often administered to these patients to treat an associated condition, secondary hyperparathyroidism. Unfortunately, calcitriol treatment may promote medial calcification. Our work aimed to determine how calcitriol and combinations of calcitriol, fibroblast growth factor 23 (FGF-23), and klotho affect vascular smooth muscle cell (VSMC) calcification. **Methods:** Human VSMCs were cultured in normal and high phosphate conditions and given three concentrations of calcitriol (10, 100, and 1000 nM). Calcium content was quantified through atomic absorption. Additionally, protein expression and surface morphology were examined of VSMCs treated with 100 nM calcitriol. Lastly, VSMCs were cultured in high phosphate and given combinations of calcitriol, FGF-23, and klotho. **Results:** Calcitriol supplementation alone increased calcification, but was not associated with a transition towards an osteoblast-like phenotype.The combination of calcitriol and FGF-23 caused a decrease in calcification, but the combination of all three increased calcification. **Conclusions:** Calcitriol alone increased calcification, but combinations of calcitriol, FGF-23, and klotho caused differential effects, showing the importance of this interaction to the process of medial calcification and may help explain the variable results found in previous research.

Keywords: Calcitriol; chronic kidney disease; fibroblast growth factor 23; klotho; medial calcification; human vascular smooth muscle cells

INTRODUCTION

Medial calcification, or Mönckeberg's arteriosclerosis, is the pathological deposition of calcium-phosphate mineral along the elastic fibers in the middle layer of arteries and is associated with chronic kidney disease (CKD), diabetes, and ageing. It causes increased arterial stiffness and is correlated with an increased risk of total and cardiovascular mortality in type 2 diabetes patients [1] and CKD patients on hemodialysis [2]. It is now widely believed to be an active process that involves four key events: 1) the trans-differentiation of Vascular Smooth Muscle Cells (VSMCs) into osteoblast-like cells, 2) the release of matrix vesicles, 3) the loss of calcification inhibitors, and 4) the degradation of the extracellular matrix [3]. High concentrations of calcium and phosphate play a large role in the pathogenesis, as they are able to increase calcification in a concentration-dependent and synergistic manner [4-6].

Under normal conditions, proper mineral metabolism is maintained through the actions of the bone, kidney, and endocrine systems through the molecules vitamin D, parathyroid hormone (PTH), fibroblast growth factor 23 (FGF-23), and klotho. During CKD, patients display dysfunctional mineral metabolism, displaying decreased conversion of vitamin D to its active form 1,25-dihydroxyvitamin D (or calcitriol), increased expression of PTH (secondary hyperparathyroidism), and decreased expression of klotho. To treat secondary hyperparathyroidism, patients will often receive calcitriol supplementations. While research has shown that it does increase the survival rate of CKD patients [7], large doses of calcitriol can cause hypercalcemia and promote medial calcification. Less calcemic analogues, such as paricalcitol [8], have been created in order to avoid these side effects; however, research has been inconclusive whether calcitriol treatment increases or decreases medial calcification and whether it is a systemic or local reaction. Some studies have shown that calcitriol increases both *in vitro* and *in vivo* calcification [9-12], others have shown that it decreases calcifications [13-16], and the rest have shown mixed results [17-20]. Many of the recent studies that have shown positive results from calcitriol treatment believe it to be associated with an increase in klotho expression and subsequent reaction with FGF-23 [15, 16]. Because there are no clinically available treatments for medial calcification, understanding the mechanism behind calcitriol's effect as well as its interaction with FGF-23 and klotho could lead to the development of a potential therapy utilizing these molecules.

The purpose of this study was to: 1) observe the *in vitro* effects of 10, 100, and 1000 nM concentrations of calcitriol on VSMC calcification in the presence of normal and high phosphate, 2) examine the surface morphology and protein expression of VSMCs given 100 nM calcitriol in the presence of normal and high phosphate, and 3) observe the *in vitro* effects of combinations of calcitriol, FGF-23, and soluble klotho on VSMC calcification in the presence of high phosphate.

SUBJECTS AND METHODS

Materials

Calcitriol was obtained from Tocris Biosciences. Recombinant human klotho and FGF-23 were obtained from R&D Systems.

For western blot analysis, anti-alkaline phosphatase (ALP), anti-α-smooth muscle actin (αSMA), anti-smooth muscle myosin heavy chain (SM-MHC), and anti-klotho antibodies were obtained from Abcam. Anti-ERK 1 and anti-ERK 2 antibodies were obtained from Santa Cruz Biotechnology.

Cell Culture

Human primary aortic VSMCs were purchased from ATCC. Cells were grown and maintained using Dulbecco's Modified Eagles Medium with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were incubated at 37ºC with 5% CO_2, and media was changed every 2-3 days.

For the experiments, the HVSMCs were seeded in cell culture plates and allowed to grow to ~80% confluency. Upon reaching ~80% confluency (day 0), each group received the different treatments for 7 or 14 days, with the media being changed every 2-3 days. During this time, all media contained 10% charcoal-stripped FBS in place of regular FBS to remove residual vitamin D metabolites. For experiments, normal phosphate (NP) groups refers to groups receiving standard cell culture media, and high phosphate (HP) groups refers to groups receiving media supplemented with 3 mM inorganic phosphate in the form of dibasic sodium phosphate. Cells were at passage 8 for all experiments.

To examine concentration-dependent effects, three concentrations (10, 100, and 1000 nM) of calcitriol were added to both NP and HP media. For this experiment, cells were given the appropriate media for 14 days. For further examination of the effects of calcitriol supplementation, a concentration of 100 nM was chosen, as it was the lowest concentration that a change was observed in calcium deposition. For these experiments, cells were given the appropriate media for both 7 and 14 days. When examining the effects of the combinations of calcitriol, FGF-23, and soluble klotho, concentrations of 100 nM for calcitriol, 10 ng/mL for FGF-23, and 0.4 nM for soluble klotho were chosen and added to HP media. One group was grown in NP media with the vehicle for comparison. For this experiment, cells were given the appropriate media for 14 days.

Calcium Deposition Quantification

Media was removed from the cell cultures, and they were rinsed gently with 1x phosphate buffered saline (PBS). The cell layers were then decalcified by using 0.6 N HCl for 24 hours. HCl supernatants were collected, and the cell layers were then solubilized using 0.1 N NaOH/0.1% sodium dodecyl sulfate (SDS) solution.

Calcium concentration of HCl supernatant were determined with atomic absorption spectroscopy (AA). It was conducted using a Shimadzu AA-7000F (Shimadzu Corp) and Calcium Atomax Hollow Cathode Lamp (PerkinElmer) using a wavelength of 422.7 nM. Calcium content was normalized to intracellular protein content. Protein concentration of the NaOH/SDS solution was determined using the Pierce bicinchoninic acid (BCA) assay (Thermo Scientific) following manufacturer's instructions.

Visualization of Calcium Deposition

Visualization of the calcium deposition was done using scanning electron microscopy (SEM). Prior to seeding cells in the six well plates, Thermanox plastic coverslips were placed in wells with the treated side face-up. After receiving the appropriate media for 7 or 14 days, media was removed, and cells were fixed in 1/2 Karnovsky's fixative in 0.1 M sodium cacodylate buffer for up to two weeks. The samples were processed for SEM imaging by further fixation using 0.4% osmium tetroxide followed by serial dehydration, using increasing concentrations of ethanol and hexamethyldisilazane (HMDS). Lastly, samples were allowed to air dry overnight, mounted, and sputtered coated with 15 nm of platinum using an EMS 1150T ES sputter coater (Electron Microscopy Science). Samples were imaged using a Carl Zeiss EVO50VP Variable Pressure Scanning Electron Microscope (Zeiss).

Examination of Protein Expression

Protein was obtained by placing cells in lysis buffer (10 mM Hepes, 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 10 mM Na-pyrophosphate, 10 mM NaF, 0.1 mM Na-orthovanadate, 1% Na deoxycholate, 1% Triton x 100, and 0.1% SDS), scraped, sonicated on ice for 5 seconds, and centrifuged at 15000 RPM to remove large cellular debris. Protein concentration was determined using BCA assay and diluted such that 20 µg of protein was loaded in each well. Samples were loaded into the wells of 10% or 8% SDS-Page gel and ran at a constant voltage (120 V) for approximately 2 hours or until tracking blue dye reached the bottom. Once finished, gels were transferred to PDVF membrane overnight.

Membranes were blocked with 5% non-fat dry milk in 1x Tris-Buffered Saline with 0.05% Tween 20 (TBST) buffer for one hour at room temperature. Primary antibodies were added to 5% milk in 1x TBST buffer, solution was placed on membranes, and membranes were allowed to shake overnight at 4^o C. Membranes were rinsed with 1x TBST. Secondary antibodies were added to 1% milk with 1x TBST buffer and placed on membranes for 1-2 hours at room temperature. ECL solution was added to each membrane, allowed to incubate 5 minutes at room temperature, and developed on film.

Statistical Analysis

Data is presented as mean ± standard deviation, and error bars on graphs represent standard deviation. For all AA experiments, n = 6. For western blot and SEM, n = 3. When comparing two groups, student t-test was used (α= 0.05). When comparing multiple groups, one-way ANOVA with Fisher's Least Significant Difference (LSD) post-hoc analysis was used (α= 0.05).

RESULTS

Effects of Calcitriol Concentrations on VSMC Calcification

To examine any concentration-dependent effect of calcitriol, three concentrations of calcitriol (10, 100, and 1000 nM) were added to VSMCs in the presence of normal and high phosphate, and calcium deposition was quantified with AA. As seen in Figure 1 (left), in the NP groups, only 1000 nM calcitriol (94.29 ± 83.1 µg/mg protein) was able to cause a significant increase in calcium content compared to the vehicle (18.29 ± 20.39 µg/mg protein). In the HP groups, both 100 nM (3293.02 ± 1674.01 µg/mg protein) and 1000 nM calcitriol (2608.60 ± 1182.96 µg/mg protein) caused a significant increase in calcium content compared to the vehicle (1078.52 ± 325.92 µg/mg protein), but no significant difference was found between them. It is worth noting the calcium content value of 1000 nM NP group was still ~10 times lower than the vehicle HP group.

In-Depth Analysis of Calcitriol Supplementation on VSMC Calcification

To further analyze the effects of calcitriol supplementation on VSMC calcification, one concentration of calcitriol was chosen (100 nM). The increase in calcification of VSMCs observed in the previous experiment was confirmed using AA and observed qualitatively using SEM. Protein expression (αSMA, SM-MHC, ALP, and Klotho) was examined using western blot analysis.

The calcium content of VSMCs can be seen in Figure 1 (right). After 7 days, the supplementation of 100 nM (26.71 ± 10.62 µg/mg protein) caused a significant increase in calcification compared to the vehicle (4.08 ± 0.75 µg/mg protein) in the NP groups, while there was no effect in the HP groups (100 nM calcitriol, 166.94 ± 17.88 µg/mg protein; vehicle, 169.41 ± 54.12 µg/mg protein). On the contrary, at 14 days, we observed a significant increase in calcium content with the addition of 100 nM calcitriol in the HP groups (100 nM calcitriol, 654.66 ± 299.92 µg/mg protein; vehicle, 380.20 ± 49.62 µg/mg protein) but not the NP groups (100 nM calcitriol, 61.90 ± 6.58 µg/mg protein; vehicle, 56.17 ± 7.40 µg/mg protein).

SEM images (at 1000x magnification) after 7 and 14 days can be seen in Figure 2. Comparing the images at day 7, there appears to be more small nodules in the HP groups compared to the NP groups. Although the AA data showed that there was increase in calcification with calcitriol supplementation in the day 7 NP groups, there does not appear to be distinct morphological differences between these two groups. The images after 14 days of treatment are similar to the 7 day images, in that the HP groups appear to have more nodule formations than the NP groups. Also, while AA data showed an increase in calcification with the supplementation of 100 nM calcitriol in the 14 day HP group, there again did not appear to be any morphological differences between the two groups.

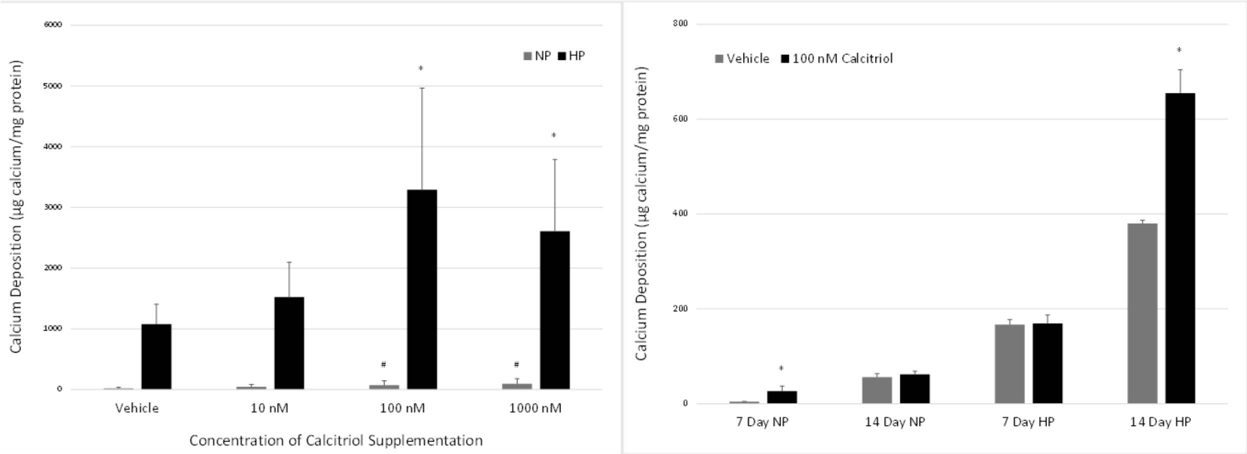

Figure 1. (Left) Effects of three concentrations of calcitriol on VSMC calcification in the presence of normal and high phosphate after 14 days. * P < 0.05 compared to HP vehicle and # P < 0.05 compared to NP vehicle by Fisher's LSD post hoc test. (Right) Effects of 100 nM calcitriol on VSMC calcification in the presence of normal and high phosphate after 7 and 14 days. * P < 0.05 compared to vehicle with same treatment by student t-test.

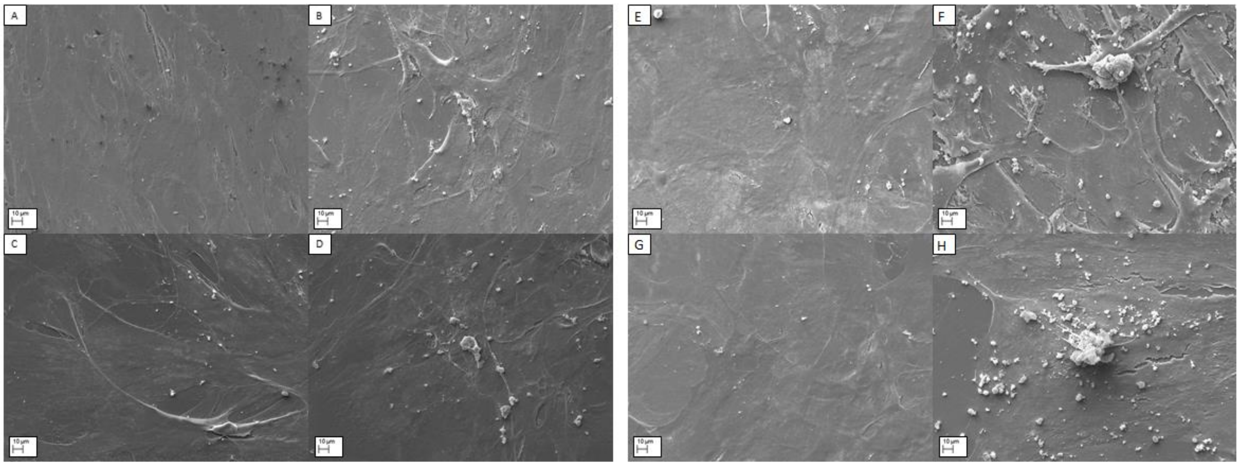

Figure 2. Representative SEM images (1000x magnification) of (A) NP vehicle, (B) HP vehicle, (C) NP 100 nM calcitriol, and (D) HP 100 nM calcitriol after 7 days of treatment, (E) NP vehicle, (F) HP vehicle, (G) NP 100 nM calcitriol, and (H) HP 100 nM calcitriol after 14 days of treatment. Scale bar is equal to 10 µm.

The protein expression of αSMA, SM-MHC, ALP, and klotho were examined using western blot analysis. The relative protein expression can be found in Figure 3. At 7 and 14 days, there was a decrease in αSMA between the NP and HP groups. However, there did not appear to be any effect in αSMA expression caused by the addition of 100 nM in either group. SM-MHC expression followed a similar trend as αSMA but to a lesser degree. There also did not appear to be any effect of phosphate or calcitriol supplementation on ALP expression. Finally, there did not appear to be any change to the total klotho expression between any of the groups. Total ERK was used as a loading control.

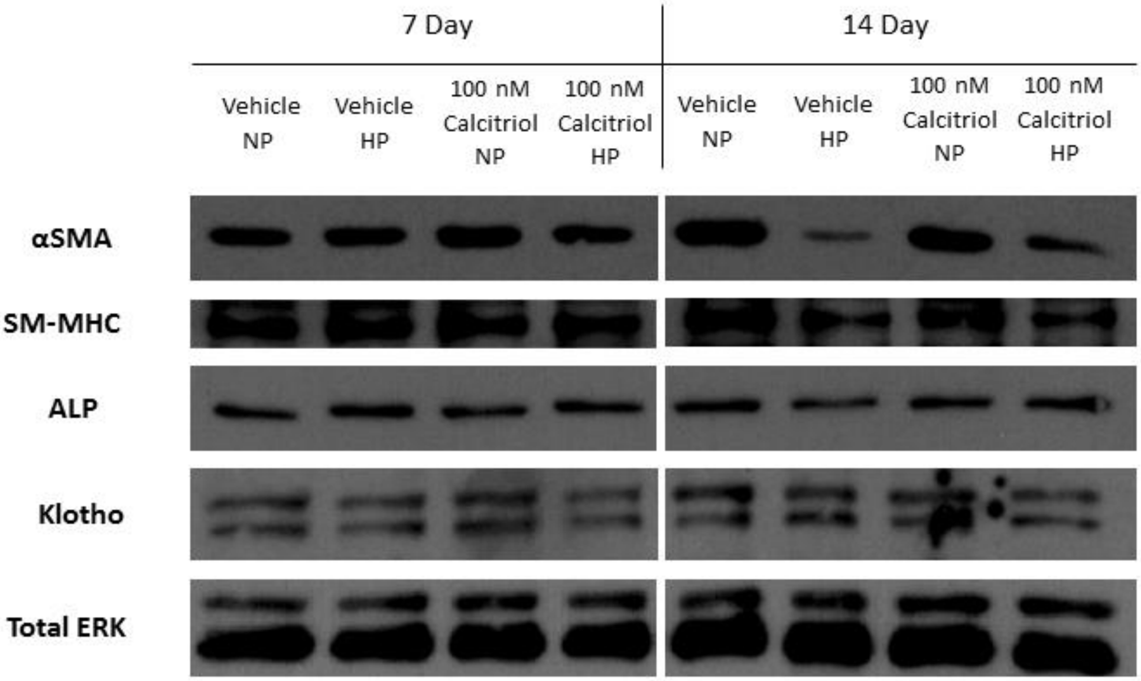

Figure 3. Relative protein expression of αSMA, SM-MHC, ALP, and klotho. Total ERK was used as loading control.

Effects of Calcitriol, FGF-23, and Klotho Supplementation on VSMC Calcification

In order to observe the effects from the interaction of calcitriol, FGF-23, and klotho, various combinations were added to VSMCs in HP conditions, and calcium content was quantified using AA. As seen in Figure 4, the addition of FGF-23 and calcitriol (29.63 ± 4.81 µg/mg protein) caused a significant decrease in calcium content compared to the vehicle (726.35 ± 537.60 µg/mg protein) and was similar to the calcium content of VSMCs that did not receive phosphate supplementation (9.18 ± 7.67 µg/mg protein). Surprisingly, the calcium content of the VSMCs that received the combination of calcitriol, FGF-23, and klotho was significantly higher than all the other groups (3406.51 ± 810.45 µg/mg protein).

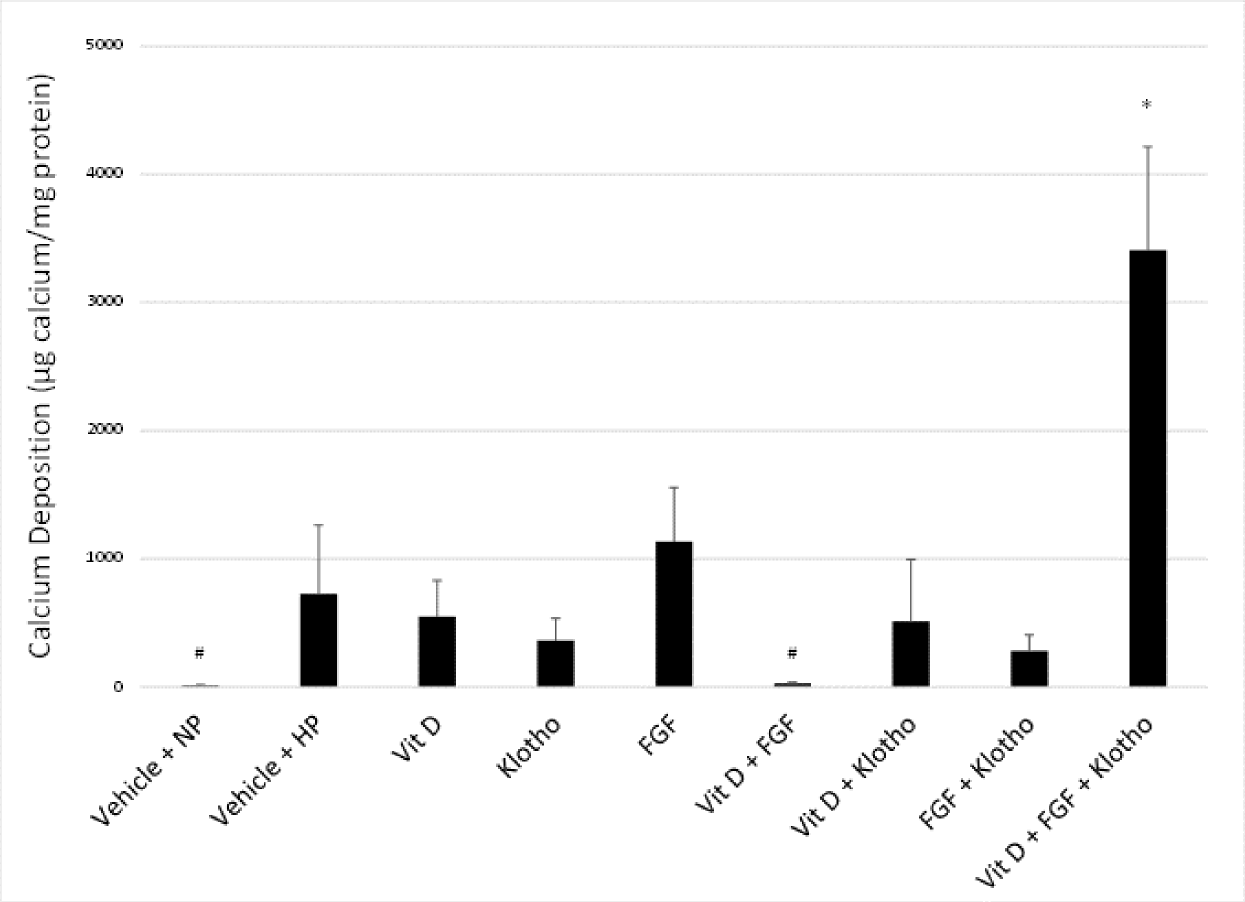

Figure 4. Effects of combinations of 100 nM calcitriol, 10 ng/mL FGF-23, and 0.4 nM soluble klotho on VSMC calcification in the presence of high phosphate after 14 days. * $P < 0.05$ compared to all groups and # $P < 0.05$ compared to HP vehicle by Fisher's LSD post hoc test.

DISCUSSION

In order to determine if calcitriol treatment promotes VSMC calcification, many *in vitro* and *in vivo* studies have been conducted; however, the results have often been contradictory. When looking at the concentration-dependent effects of calcitriol on VSMC calcification, we observed that 100 and 1000 nM calcitriol supplementation was able to increase the calcification in the presence of high phosphate and only 1000 nM calcitriol supplementation was able to increase the calcification in the presence of normal phosphate. The first study to reveal detrimental effects of calcitriol supplementation on *in vitro* VSMC calcification, published in 1998 by Jono et al, showed that calcitriol supplementation (as low as 10 nM) on bovine VSMCs increased calcification in the presence of high phosphate [9]. Another study revealed that calcitriol supplementation (100 and 300 nM) increased calcification *in vitro* rat VSMC grown with β-glycerosphosphate. It is worth noting that they did not find a difference between the calcification caused by 100 and 300 nM [11], similar to our 100 and 1000 nM HP groups. It was recently showed that calcitriol supplementation increased calcification in mouse VSMC cultures in not only high phosphate conditions, but also in normal phosphate conditions [12]. One study showed calcitriol decreased *in vitro* calcification; however, this study involved the addition of an inflammatory cytokine that accelerates calcification [14]. This, however, could be due to calcitriol's role in immune system regulation [21], as calcitriol did not have an effect on calcification caused by high phosphate alone in this study [14]. Taken altogether, it is clear that calcitriol alone does have a direct effect on the *in vitro* VSMC calcification.

Interestingly, the degree of calcification caused by 1000 nM calcitriol in normal phosphate conditions was ~10 times lower than the calcification caused by high phosphate alone. This could help explain the effects caused by FGF-

23 [18] and klotho knockout [19]. FGF-23 null mice with a normal diet had accelerated mortality and increased calcification, calcitriol, serum calcium, and serum phosphate, but low-phosphate dietary restriction resulted in no arterial calcification, despite high serum calcitriol [18]. Very similar results were found when observing klotho knockout mice. They displayed growth retardation, increased calcification, and increased serum calcium, phosphate, FGF-23, and calcitriol, but with normalization of serum phosphate levels, vascular calcification was abolished, despite the continued high serum calcium and calcitriol [19]. These results suggest that while calcitriol can have an effect with normal phosphate, high phosphate is more important to the pathogenesis of medial calcification.

When we further examined the effects of 100 nM calcitriol supplementation on the VSMCs with AA, SEM, and western blot. At 7 days, 100 nM calcitriol supplementation did not increase calcification in the presence of high phosphate but did with normal phosphate. After 14 days, 100 nM calcitriol supplementation increased calcification in the presence of high phosphate but did not in the absence of high phosphate after 14 days. A time-dependent effect has not been previously noted; however, it is worth noting that calcitriol's effects on osteoblast differentiation is highly dependent on the stage of maturation of the cell [22]. In fact, recent research has shown that calcitriol accelerates matrix vesicle formation in osteoblasts but only during the early phase of differentiation [23]. Thus, it is possible that calcitriol has a similar stage-dependent effect on VSMCs, but more research needs to be conducted before any conclusion can be reached. Looking at the SEM images, nodule formations appear in large number in the calcification groups but not the control groups, suggesting that these are the mineral deposits. Comparing the vehicle groups to the 100 nM calcitriol groups, there do not appear to be any distinct morphological differences to suggest a cause for the increase in calcification caused by the calcitriol supplementation. To the best of our knowledge, we are the first group to observe calcifying VSMCs using SEM. When looking at protein expression, we observed a loss of αSMA and SM-MHC expression in the calcification groups compared to the control groups at 7 and 14 days, consistent with previous studies that show that VSMCs deposit mineral and undergo transition to an osteoblast-like phenotype with the addition of high phosphate [6]. On the other hand, there did not appear to be a difference in αSMA and SM-MHC expression between the vehicle and 100 nM calcitriol groups at either 7 or 14 days. Han et al showed an increase in runx2 expression after the supplementation of 100 nM calcitriol [12]. While we were unable to detect runx2 expression in our cells (data not shown), upregulation of runx2 is typically coupled with the loss of smooth muscle markers, so it is surprising that we did not see a difference in αSMA or SM-MHC. Our results actually suggest that the increase in calcification caused by calcitriol supplementation may be independent of the phenotypic change.

Lastly, we examined the effects of combinations of calcitriol, FGF-23, and soluble klotho. We saw a significant decrease in calcification with the combination of calcitriol and FGF-23, agreeing with the findings of Lim et al. In their study, this decrease is due to the increased expression of transmembrane klotho, as klotho siRNA was able to abolish this decrease in their study [16]. It is believed that the increased expression of transmembrane klotho allows FGF-23 to react with its receptor and exert a beneficial effect. Soluble klotho alone is believed to be able to elicit a positive effect, as Hu et al showed that the addition of 0.4 nM soluble klotho decreased VSMC calcification *in vitro* [24]. In their experiment, they added 2 mM inorganic phosphate, so it is possible that 0.4 nM was too low of a concentration to elicit a similar, beneficial effect on the VSMC calcification caused by 3 mM inorganic phosphate in our study. The most surprising result was that the addition of all three, calcitriol, FGF-23, and soluble klotho, caused an extreme increase in VSMC calcification. This is the first time this combination has been added to VSMCs in the presence of high phosphate *in vitro*. Research has shown that soluble klotho can still act as a coreceptor for FGF-23 signaling [25], although it is believed to not be as active as the membrane form. Because the FGF-23 + calcitriol group and the FGF-23 + klotho group do not experience an increase in calcification, it may be possible that the combination of calcitriol, FGF-23, and klotho allows for excess FGF-23 activity. Supporting this idea, Jimbo et al found that FGF-23 causes concentration-dependent increase of calcification in klotho-overexpressing VSMCs in the presence of high phosphate [26]. While more research needs to be conducted to determine the mechanism behind this interaction, it is clear that calcitriol's effect on VSMC calcification is complicated and involves interactions with the other endocrine molecules, FGF-23 and klotho. This, in part, may help explain the contradictory results found both *in vitro* and *in vivo* research.

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Conflict of Interest: KAB, None to declare; CLS, None to declare

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Plant Density Effect of Organic Eggplant (*Solamun melongena* L) on Yield, Biomass Development and Soil Loss Prediction

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ABSTRACT

As a part of the conservation research being carried out on vegetable crops in Mississippi, a study was conducted on eggplant (Solanum melongena L. 'Black Beauty') to determine the effects of plant density on yield, biomass development and soil loss prediction at various growth stages. Two plots were used on a Memphis silt loan soil (Typic Hapludalf, silty, mixed, and thermic) at low plant density (LPD), 0.760 m x 0.912 m; and high plant density (HPD), 0.609 m x 0.912 m in the summer of 2005. No fertilizers were applied to the crop. All plants received equal quantity of manures with 0.454 g of worm castings and composed cow manure. Three plants from each experimental unit in four replications (for a total of 12) were randomly selected for destructive harvest at various growth stages. A pair completely randomized design was used and analysis of variance was conducted. Plant and canopy height, rhizosphere width, and root length were higher for HPD compared to LPD. Leaf area index, percent canopy cover, dry upper and total biomass per plant, fruit length and diameter, and yield were higher for LPD. There was no difference in canopy width and stem diameter. LPD is more advisable for farmers because it was higher in leaf area index, total dry biomass, which indicates a higher degree of soil protection, and yield per plant.

Keywords. Eggplant, organic, plant density, total dry biomass and yield.

INTRODUCTION

Farmers have been changing and adopting new technologies to make their production safer and organic agriculture is one of them. Indeed, organic agriculture has become very important due to increased concern on environmental quality. In fact, ground water and surface water supplies are threatened with contamination. Crop nutrients from agricultural fertilizers are the most serious and widespread source of excess N and P (National Research Council, 1993). Because of these facts farmers are willing to accept organic farming as an alternative to inorganic agriculture. Most importantly, the total sales of organic products reached a value of $ 1.82 billion and the land used for their production was 1.28 million acres (USDA-National Agricultural Statistical Service, 2007) Moreover, about 800 new organic products were introduced in the first half of 2000 (Dimitri and Greene, 2000). Additionally, the practice of organic agriculture can improve soil physical, chemical and biological properties, promote species diversity, reduce surface and ground water pollution, protect people's health, and enhance the nutritional value of fruit and vegetables (Eghball and Power, 1999; Gao and Chang, 1996; Arriaga and lowery, 2003; Ndayegamiye and Cote, 1989; Organic Trade Association, 1999; Assami et al., 2003; Carbonaro et al., 2002).

Soil erosion is a major conservation issue on about 50% of US cropland (Larson, 1981). Previous studies by Benbrook et al.(1984) have estimated the on farm costs of soil erosion in the US at between $525 million and $1 billion per year. In the US, up to a billion tons of agricultural soils are deposited in waterways every year, and an estimated one-half of the sediments in the water originated from agriculture (Organization for Economic Cooperation and Development, 1994).

Mississippi presents serious erosion problems. Ribaudo (1992) has estimated the cost of this impairment to be about $ 9 billion per year. Specifically, the Mississippi delta area has serious soil erosion problem despite relatively flat slopes (Murphree and Mutchler, 1981; Cooper and Knight, 1990).

Due to erosion problems, The Universal Soil Loss Equation (USLE) was developed by the National

Runoff and Soil Loss Data Center. The creation of the USLE brought a new era in the prediction of soil erosion (Peterson and Swan, 1979).

A general description of the USLE is given below:

$$A= R.K.LS.C.P$$

Where A is the average annual soil loss, the factor R represents effects of climatic erosivity; K, soil erodibility; LS, slope length and steepness; and C, cover and management; and P, supporting conservation practices. The C-factor has the strongest effect on the model. (Risse et al., 1993)

The C-factor is the ratio of soil loss from land cropped under specific conditions to the corresponding loss from tilled, continuous fallow conditions. The dimensionless C-factor, which has a range between 0 and 1, indicates the degree of protection of the soil surface by the crop or vegetation (Biesemans et al., 2000).

Of the five factors in USLE, the cover and management (C) factor is the most important one from the standpoint of conservation because land use changes meant to reduce erosion are represented here (Panicker et al., 2001; Panicker et al., 2004).

Eggplant (*Solanum melongena* L.) represents an important vegetable crop in the world. The yield of this crop in the world in 2014 was 268308 kg/ha and its value \$50,19 millions (FAO, 2014). Its production value in the US at \$ 4,18 millions in 2001 and with 6038 acres planted in 2007. (USDA-NASS, 2004; USDA-NASS, 2007).

The parameters mentioned above are needed to estimate erosion loss in eggplant fields at different plant population using USLE program. Since these values are absent from the Agriculture Research Service (ARS) and Soil Conservation Service (SCS) database, this study was undertaken to assist in the collection of the sub factor values of the C-factor to be used in the model for estimating soil loss from eggplant fields, in addition to other set objectives. The specific objectives of this study were: To predict possible soil loss based on sub factor values of the crop management factor (C-Factor) of the Universal Soil Loss Equation (USLE), and RUSLE and determine the influence of eggplant density on biomass development and fruit yield in Mississippi.

MATERIALS AND METHODS

Cultural practices. Experiments were conducted at the conservation research project at Alcorn State University, Mississippi whose coordinates are N 31^0 54''; W 91^0 09'', between 6 June. 2005 and 16 Nov. 2005. The soil was Memphis Silt Loan Soil (Typic Hapludalf, silty, mixed, thermic).

Seeds of eggplant *(Solanum melongena* L. 'Black Beauty') were planted and its seedlings were grown for 45 days in a greenhouse. Prior to planting, the soil was prepared by performing the following activities: disked once, tilled with rotary tiller 10 times, and raised beds with middle buster.

Two experimental plots were simultaneously established and maintained for the same crop; low plant density (LPD) of 0.76 m x 0.912 m and another high plant density (HPD) of 0.609 m x 0.912 m. Also, each plot was divided into two sections; one for destructive harvest studies and the other for nondestructive yield harvest studies. The area selected for the plots was fallowed for 8 years prior to the experiment. Three plants from each experimental unit were randomly selected for destructive harvest at various growth stages.

Plant measurements. Destructive harvest was carried out for every 20 days from the date of planting until the final harvest. The variables measured at each destructive harvest were: leaf area index (LAI), percent canopy cover, canopy height (it is the distance from soil surface to the tip of the highest leaf on the plant), canopy width (measurement of the canopy of the plant from side to side), stem diameter (measurement across the stem 10 cm above soil surface), plant height (measurement of the plant from soil surface to the terminal bud), root width (measurement of the root from side to side in the soil), root length (measurement of the root from its origin in the stem to the deepest point into the soil), root mass 0-10 cm depth (dry weight of roots), root mass below 10 cm depth (dry weight of roots),upper biomass (dry weight of shoots). For the non-destructive studies, fruits were harvested at maturity and the following variables were measured: fruit diameter (measurement across the fruit), fruit length (measurement of the fruit from bottom to top, total dry root biomass (weight of roots after drying), total dry biomass (dry root weight plus shoots) and yield.

Zenith angle. Zenith angle is the angle the sun makes with respect to a line vertical to the earth's surface. Zenith angle of the sun is required for inversion of canopy light transmission data to determine leaf area index (AccuPAR Operator's Manual, 1995). The zenith angle was determined with a device called board/scale zenith angle device before the ceptometer reading is taken.

Leaf area index (LAI). LAI is the area of leaves per unit area of soil surface. LAI and percent canopy cover are measured with the AccuPAR. AccuPAR is a battery-operated linear PAR ceptometer used for the collection of light interception data in crop and forest canopy research. AccuPAR's sensors measure PAR (Photosynthetically Active Radiation) in the 400 to 700 nanometer waveband (AccuPAR Operator's Manual, 1995).

AccuPAR reading was taken between 10:00 a.m. and 2:00 p.m. The AccuPAR was first set in READ mode F2, then use F4 for segmented probe par sampling with no external point sensor. The unshaded F1 and one reading shaded F4. By pressing the F4 key, the probe automatically calculated 12 readings and averaged them.

To minimize the chances of errors while taking readings inside the plot, the border rows of the undisturbed yield harvest area were avoided. By the same procedure mentioned above, one reading was taken above the canopy with a F1 key. Next, by pressing the F4 key 1 time, the AccuPAR automatically calculated and averaged 12 readings below the canopy. The center of the probe was placed close to the stem of the plant and moved slowly away as the AccuPAR calculated readings.

Percent canopy **cover**. Canopy cover percent is the surface of the soil covered by the canopy expressed in percentage. The instrument was set up on function 2 and placed above the leaf canopy in full sunlight and then, button A was pressed. Border rows were avoided to record this reading. This D_v is the measurement when the AccuPAR probe is fully exposed to sunlight. The B button was pressed twice to clear the display. After taking 10 readings below the canopy press the button B was pressed to display the arithmetic mean $N_{um.}$ Percent Canopy Cover (PCC) = $(1 - N_{um} \div D_v) \times 100$. (AccuPAR Operator's Manual, 1995).

Root mass 0-10 cm depth (dry weight roots) is a variable that is important for erosion prediction models due to the fact that the majority of the roots in most of the crops are located in the first 10 cm and they hold the soil firmly, so they keep the soil from being eroded.

Height, length and width measurements of plants and fruits were taken with a ruler. Diameter measurements of stems and fruits were taken with a caliper and finally, weight measurements of shoots, roots and fruits were taken with a scale. A completely randomized design was used and analysis of variance (ANOVA) was conducted at 5 % significant level by using the statistical analysis system (SAS).

RESULTS

Leaf area index. There were significant differences between the treatments for the first harvest (Table 1). In other words, plants in the LPD treatment produced more leaf area per unit of ground area than plants in HPD treatment. Indeed, LPD treatment presented the highest value with 0.046 whereas HPD treatment produced the lowest value with 0.026. For the second harvest, there were no significant differences between the treatments. Moreover, HPD treatment had the highest value with 0.09 while LPD treatment presented the lowest value with 0.08. For the third harvest, no significant differences between the treatments were detected. Also, LPD treatment produced the highest value with 0.17 and in turn, HPD treatment had the lowest value with 0.16. For the fourth harvest, there were no significant differences between the treatments. In addition, LPD treatment presented the highest value with 0.39 and on the other hand, HPD treatment presented the lowest value with 0.34. No significant differences were found for the fifth harvest. Additionally, LPD treatment produced the highest value with 0.27 while HPD treatment presented the lowest value with 0.25. Lastly, there were no significant differences between the treatments for the sixth harvest. In fact, LPD treatment presented the highest value with 0.25 whereas HPD treatment produced the lowest value with 0.17 (Table 1).

Destructive harvest

Average leaf area index in six stages of growth period

Treatments	I	II	III	IV	V	VI
Low plant density	0.1	0.7	4.3	16.2	5.7	10.6
High plant density	0.08	0.6	2.3	18.3	6.4	8.6
Least significant difference 5%	0.48**	0.37 NS	5.67 NS	22.29 NS	2.42 NS	4.56 NS
Average percent canopy cover in six stages of growth period						
Low plant density	11	19.6	29.4	50.8	45.4	54.4
High plant density	7.3	17.8	27.1	44	39.2	55.4
Least significant difference 5%	3.50**	9.32 NS	6.52 NS	12.63 NS	10.99 NS	8.02 NS
Average plant height (cm) in six stages of growth period						
Low plant density	17.9	15	37	52.2	60.3	67.2
High plant density	23.1	20.1	31.8	50.6	63.8	79.2
Least significant difference 5%	7.66 NS	5.52 NS	6.21 NS	7.74 NS	9.38 NS	7.15 NS
Average canopy height (cm) in six stages of growth period						
Low plant density	27.5	29.9	45.5	60.4	68.4	72
High plant density	23.3	33.2	41.6	55.2	70.1	74.8
Least significant difference 5%	4.12**	5.76 NS	7.47 NS	9.73 NS	11.35 NS	7.48 NS
Average canopy width (cm) in six stages of growth period						
Low plant density	36	44.4	57.5	79	79.8	77.7
High plant density	21.5	42.9	59.1	74.3	75.1	76.6
Least significant difference 5%	4.22**	8.44 NS	9.09 NS	10.86 NS	11.02 NS	9.41 NS
Average rhizosphere width (cm) in six stages of growth period						
Low plant density	23.4	34.6	60.4	94.8	111.3	109.6
High plant density	16.1	34.4	51.9	100.6	118.7	114.7
Least significant Difference 5%	3.23**	3.85 NS	7.31**	7.72 NS	6.87**	5.97 NS

**Significant at 5%; NS: non-significant

**significant

Percent canopy cover. There were significant differences between the treatments for the first harvest (Table 2). That is, LPD treatment produced plants with bigger canopies that consequently cover more soil surface that plants in HPD. In fact, LPD presented the highest value with 11% while HPD produced the lowest value with 7.3%. No significant differences between the treatments were detected for the second harvest, having LPD treatment the highest value with 19.6% whereas HPD treatment presented the lowest value with 17.8%. For the third harvest, no significant differences between the treatments were found. In addition, LPD treatment presented the highest value with 29.4% and in turn, HPD treatment presented the lowest value with 27.1%. For the fourth harvest, there were no significant differences between the treatments. Moreover, LPD treatment presented the highest value with 50.8% and on the other hand, HPD treatment presented the lowest value with 44%. For the fifth harvest, no significant differences were detected between the treatments. Also, LPD treatment produced the highest value with 45.4% whereas HPD treatment presented the lowest value with 39.2%. Finally, there were no significant differences between the treatments at for the sixth harvest. Additionally, HPD treatment had the highest value with 55.4% while LPD treatment presented the lowest value with 54.4% (Table 2).

Plant height .There were no significant differences between the treatments (Table 3). As a matter of fact, high plant density (HPD) presented the higher value with 23.1 cm and low plant density (LPD) presented the lowest value with 17.9 cm. For the second harvest, there were no significant differences between the treatments. However, HPD treatment presented the highest value with 20.1 cm and LPD treatment had the lowest value with 15 cm. For the third harvest, there were no significant differences between the treatments. Also, LPD treatment presented the highest value with 37 cm whereas HPD treatment produced the lowest value with 31.8 cm. For the fourth harvest, there were no significant differences between the treatments. Further, LPD treatment produced the highest value with 52.2 cm and on the contrary, HPD treatment presented the lowest value with 50.6 cm. For the fifth harvest, there were no significant differences between the treatments. Additionally, HPD treatment had the highest value with 63.8 cm while LPD treatment presented the lowest value with 60.3 cm. Finally, there were no significant differences between the treatments for the sixth harvest. In addition, HPD treatment presented the highest value with 79.2 cm and in turn, LPD presented the lowest value with 67.2 cm .

Canopy height. There were significant differences between the treatments for the first harvest (Table 4). In fact, LPD treatment had plants with taller canopies with an average value of 27.5 cm whereas HPD treatment presented plants with smaller canopies with an average value of 23.3 cm. for the second harvest, there were no significant differences between the treatments, having HPD treatment the highest value with 33.2 cm while LPD treatment produced the lowest value 29.9 cm. For the third harvest, there were no significant differences between the treatments. Additionally, LPD treatment had the highest value with 45.5 cm and on the other hand, HPD treatment presented the lowest value with 41.6 cm. For the fourth harvest, there were no significant differences between the treatments. In addition, LPD treatment had the highest value with 60.4 cm and in turn, HPD treatment produced the lowest value with 55.2 cm. For the fifth harvest, no significant differences were detected between the treatments, presenting HPD the highest value with 70.1 cm whereas LPD produced the lowest value with 68.4 cm. Lastly, for the sixth harvest, no significant differences between the treatments were found. Moreover, HPD treatment had the highest value with 74.8 cm and on the other hand, LPD treatment presented the lowest value with 72 cm.

Canopy width. There were significant differences between the treatments for the first harvest (Table 5). In other words, LPD treatment produced plants with a wider canopy than HPD treatment with an average value of 36 cm whereas plants in the HPD treatment presented an average value of 21.5 cm. For the second harvest, there were no significant differences between the treatments. In addition, LPD treatment had the highest value with 44.4 cm and on the other hand, HPD treatment presented the lowest value with 42.9 cm. For the third harvest, no significant differences between the treatments. Additionally, HPD treatment produced the highest value with 59.1 cm and LPD treatment presented the lowest value with 57.5 cm. For the fourth harvest, no significant differences between the treatments were found. Moreover, LPD treatment had the highest value with 79 cm while HPD treatment had the lowest value with 74.3 cm. For the fifth harvest, there were no significant differences between the treatments. Furthermore, LPD treatment presented the highest value with 79.8 cm and in turn, HPD treatment produced the lowest value with 75.1 cm. Finally, for the sixth harvest, there were no significant differences between the treatments. LPD presented the highest value with 77.7 cm and HPD the lowest value with 76.6 cm.

Table 2: **Variables measured at different stages of growth**

Destructive harvest						
Average root length (cm) in six stages of growth period						
Treatments	**I**	**II**	**III**	**IV**	**V**	**VI**
Low plant density	21.5	31.7	41.8	60.1	82.9	94.3
High plant density	19.9	29.5	40.9	60.4	84.7	99.1
Least significant difference 5%	3.51**	5.93 NS	8.69 NS	7.83 NS	8.53 NS	5.60 NS
Average stem diameter (cm) in six stages of growth period						
Low plant density	0.7	0.9	1.2	1.5	1.9	1.8
High plant density	0.6	0.8	1	1.6	1.8	1.7
Least significant difference 5%	0.12**	0.12 NS	0.15**	0.28 NS	0.28 NS	0.18 NS
Average upper dry biomass (g)in six stages of growth period						
Low plant density	7.2	15.5	48.3	108.9	146.3	109.1
High plant density	4.5	17.8	32.7	87.5	104.5	92.5
Least significant difference 5%	1.41**	5.07 NS	15.09 NS	46.32 NS	11.02 NS	36.65 NS
Average root mass (g) from 0-10 cm depth in six stages of growth period						
Low plant density	1.2	2.2	8.11	23.2	22.8	20.5
High plant density	0.9	1.6	5.36	26.7	29.9	18.1
Least significant difference 5%	0.44 NS	1.05 NS	11.79 NS	7.32 NS	61.39 NS	36.37 NS
Average root mass (g) below 10 cm depth (dry weight) in six stages of growth period						
Low plant density	0.1	0.7	4.3	16.2	5.7	10.6
High plant density	0.08	0.6	2.3	18.3	6.4	8.6
Least significant difference 5%	0.48**	0.37 NS	5.67 NS	22.29 NS	2.42NS	4.56 NS

**Significant at 5%; NS: non-significant

Table 3: **Effect of plant densities on fruit length, fruit diameter, yield, total dry root biomass and total dry biomass**

	Variables				
Treatments	**Fruit length (cm)**	**Fruit diameter (cm)**	**Yield (kg/plant)**	**Total dry root biomass (g)**	**Total dry biomass (g)**
Low plant density	13.4	8.3	1.13	31.2	140.31
High plant density	10.4	7.6	0.74	26.7	119.19
Least significant difference 5%	5.47 NS	0.75 NS	0.32 NS	10.66 NS	23.45 NS
NS: non-significant					

Rhizosphere width. There were significant differences between the treatments at for the first harvest (Table 6). That is, roots of the plants in LPD treatment grew wider in the soil than HPD treatment with an average value of 23.4 cm whereas plants in HPD treatment presented an average value of 16.1 cm. For the second harvest, there were no significant differences between the treatments, having LPD treatment the highest value with 34.6 cm whereas HPD treatment produced the lowest value with 34.4 cm. for the third harvest, there were significant differences between the harvests. In other words, roots of plants in LPD treatment grew wider in the soil than roots in HPD treatment. In the case, LPD treatment had the highest value with 60.4 cm whereas HPD presented the lowest value with 51.9 cm. For the fourth harvest, no significant differences were detected between the treatments. Additionally, HPD treatment had the highest value with 100.6 cm while LPD treatment produced the lowest value with 94.8 cm. For the fifth harvest, there were significant differences between the treatments. That is, roots of plants in HPD treatment grew wider in the soil than roots of plants in LPD. In this case, HPD treatment presented the highest value with 118.7 cm whereas LPD treatment produced the lowest value with 111.3 cm. Finally, no significant differences between the treatments were detected at for the sixth harvest. In addition, HPD treatment produced the highest value with 114.7 cm and in turn, LPD presented the lowest value with 109.6 cm.

Table 4: Average canopy height (cm) in six stages of growth period

	Destructive harvest					
Treatments	**I**	**II**	**III**	**IV**	**V**	**VI**
LPD	27.5	29.9	45.5	60.4	68.4	72
HPD	23.3	33.2	41.6	55.2	70.1	74.8
LSD 5%	4.12**	5.76 NS	7.47 NS	9.73 NS	11.35 NS	7.48 NS

**Significant

Table 5: Average canopy width (cm) in six stages of growth period.

	Destructive harvest					
Treatments	**I**	**II**	**III**	**IV**	**V**	**VI**
LPD	36	44.4	57.5	79	79.8	77.7
HPD	21.5	42.9	59.1	74.3	75.1	76.6
LSD 5%	4.22**	8.44 NS	9.09 NS	10.86 NS	11.02 NS	9.41 NS

** Significant

Table 6: Average rhizosphere width (cm) in six stages of growth period.

	Destructive harvest					
Treatments	**I**	**II**	**III**	**IV**	**V**	**VI**
LPD	23.4	34.6	60.4	94.8	111.3	109.6
HPD	16.1	34.4	51.9	100.6	118.7	114.7
LSD 5%	3.23**	3.85 NS	7.31**	7.72 NS	6.87**	5.97 NS

** Significant

Root length. There were no significant differences between the treatments for the first harvest (Table 7). Moreover, LPD treatment presented the highest value with 21.5 cm whereas HPD treatment produced the lowest value with 19.9 cm. For the second harvest, no significant differences were found between the treatments. Additionally, LPD treatment had the highest value with 31.7 cm while HPD treatment presented the lowest value with 29.5 cm. For the third harvest, no significant differences between the treatments were detected, having LPD treatment the highest value with 41.8 cm and on the other hand, HPD treatment produced the lowest value with 40.9 cm. For the fourth harvest, there were no significant differences. In addition, HPD treatment had the highest value with 60.4 cm and on the contrary, LPD treatment produced the lowest value with 60.1 cm. For the fifth harvest, no significant differences were detected between the treatments. Furthermore, HPD treatment presented the highest value with 84.7 cm while LPD treatment produced the lowest value with 82.9 cm. Lastly, there were no significant differences between the treatments for the sixth harvest. Also, HPD treatment presented the highest value with 99.1 cm whereas LPD treatment had the lowest value with 94.3 cm.

Stem diameter. There were significant differences between the treatments for the first harvest (Table 8). In other words, plants in LPD treatment presented thicker stems than plants in the HPD treatments. Following that, LPD treatment presented the highest value with 0.7 cm while HPD treatment produced the lowest value with 0.6 cm. For the second harvest, there were no significant differences between the treatments. Also, LPD treatment produced the highest value with 0.9 cm and on the contrary, HPD treatment had the lowest value with 0.8 cm. For the third harvest, there were significant differences between the treatments. That is, plants in LPD treatment presented thicker stems than plant in HPD. In fact, LPD treatment produced the highest value with 1.2 cm and In turn, HPD treatment had the lowest value with 1 cm. For the fourth harvest, no significant differences were detected between the treatments. In addition, HPD treatment presented the highest value with 1.6 cm and on the other hand, LPD treatment produced the lowest the lowest with 1.5 cm. For the fifth harvest, no significant differences between the treatments were found. Further, LPD treatment produced the highest value produced the highest value with 1.9 cm whereas HPD treatment had the lowest value with 1.8 cm. Finally, there were no significant differences between the treatments for the sixth harvest. Moreover, LPD treatment had the highest value with 1.8 cm while HPD treatment presented the lowest value with 1.7 cm.

Dry upper biomass. There were significant differences between the treatments for the first harvest (Table 9). In other words, plants in the LPD treatment produced more dry matter in their upper biomass than plants in the HPD treatment. In this case, LPD treatment presented the highest value with 7.2 g while HPD treatment had the lowest value with 4.5 g. For the second harvest, no significant differences were detected between the treatments. In addition, HPD treatment produced their highest value with 17.8 g whereas LPD had the lowest value with 15.5 g. For the third harvest, there were significant differences between the treatments. LPD treatment produced more upper dry biomass with a value of 48.3 g compared to HPD treatment with 32.7 g. For the fourth harvest, there were no significant differences between the treatments, having HPD the highest value with 108.9 g while LPD presented the lowest value with 87.5 g. For the fifth harvest, no significant differences were detected between the treatments. Moreover, LPD treatment presented the highest value with 146.3 g while HPD treatment presented the lowest value with

104.5 g. Finally, there were no significant differences between the treatments for the sixth harvest. Furthermore, HPD treatment presented the highest value with 109.1 g and on the contrary, LPD treatment presented the lowest value with 92.5 g.

Table 7: Average root length (cm) in six stages of growth period.

	Destructive harvest					
Treatments	**I**	**II**	**III**	**IV**	**V**	**VI**
LPD	21.5	31.7	41.8	60.1	82.9	94.3
HPD	19.9	29.5	40.9	60.4	84.7	99.1
LSD 5%	3.51 NS	5.93 NS	8.69 NS	7.83 NS	8.53 NS	5.60 NS

Table 8: Average stem diameter (cm) in six stages of growth period.

	Destructive harvest					
Treatments	**I**	**II**	**III**	**IV**	**V**	**VI**
LPD	0.7	0.9	1.2	1.5	1.9	1.8
HPD	0.6	0.8	1	1.6	1.8	1.7
LSD 5%	0.12 **	0.12**	0.15**	0.28 NS	0.28 NS	0.18 NS

**Significant

Table 9: Average upper dry biomass (g) in six stages of growth period.

	Destructive harvest					
Treatments	**I**	**II**	**III**	**IV**	**V**	**VI**
LPD	7.2	15.5	48.3	108.9	146.3	109.1
HPD	4.5	17.8	32.7	87.5	104.5	92.5
LSD 5%	1.41**	5.07 NS	15.09**	46.32 NS	11.02 NS	36.65 NS

**Significant

Root mass below 10 cm depth (dry weight). There were significant differences between the treatments for the first harvest (Table 11). In other words, plants in the HPD treatment produced more roots with more dry matter than plants in the LPD treatment. In this case, HPD treatment presented the highest value with 0.08 g while the LPD treatment had the lowest value with 0.1 g. For the second harvest, there were no significant differences between the treatments. Additionally, LPD treatment had the highest value with 0.7 g whereas HPD treatment produced the lowest value with 0.6 g. For the third harvest, no significant differences between the treatments were found. In addition, LPD treatment produced the highest value with 4.3 g and in turn, HPD treatment had the lowest value with 2.3 g. For the fourth harvest, no significant differences between the treatments were detected. Moreover, HPD treatment produced the highest value with 18.3 g and on the other hand, LPD treatment produced the lowest value with 16.2 g. For the fifth harvest, no significant differences between the treatments were detected, having HPD treatment the highest value with 6.4 g while LPD treatment produced the lowest value with 5.7 g. Lastly, there were no significant differences between the treatments for the sixth harvest. Furthermore, LPD treatment presented the highest value with 10.6 g whereas HPD treatment presented the lowest value with 8.6 g.

Fruit length. There were no significant differences between the treatments (Table 12). Moreover, fruit length was higher for LPD with an average value of 13.4 cm and on the other hand, it was lower for HPD treatment with an average value with 10.4 cm.

Fruit diameter. There were no significant differences between the treatments (Table 12). In addition, fruit diameter was higher for LPD with an average value of 8.3 cm whereas it was lower for HPD treatment with an average value of 7.6 cm.

Yield. There were no significant differences between the treatments (Table 12). Furthermore, yield was higher for LPD treatment with an average value of

1.13 kg.plant^{-1} and on the other hand, it was lower for HPD treatment with an average value of 0.74 kg.plant^{-1}. Also, yield per area was higher for LPD with value of 16.291 kg.ha^{-1} and lower for HPD with value of 13.309 kg.ha^{-1}. On the contrary, total dry biomass left over after the final harvest was higher for HPD with an average value of 2.143kg ha^{-1} and lower for LPD with an average value of 2.020 kg ha^{-1}.

Total dry root biomass. There were no significant differences between the treatments (Table 12). Additionally, total dry root biomass was higher for LPD treatment with an average value of 31.2 g while it was lower for HPD treatment with an average value of 26.7 g.

Total dry biomass (upper biomass dry weight + root dry weight). There were no significant differences between the treatments (Table 12). Additionally, total dry biomass was higher for LPD treatment with an average value of 140.31 g, while it was lower for HPD treatment with an average value of 119.19 g.

Table 10: **Average root mass (g) from 0-10 cm depth (dry weight) six stages of growth period.**

	Destructive Harvest					
Treatments	**I**	**II**	**III**	**IV**	**V**	**VI**
LPD	1.2	2.2	8.11	23.2	22.8	20.5
HPD	0.9	1.6	5.36	26.7	29.9	18.1
LSD 5%	0.44 NS	1.05 NS	11.79 NS	7.32 NS	61.39 NS	36.37 NS

Table 11: **Average root mass (g) below 10 cm depth (dry weight) in six stages of growth period.**

	Destructive harvest					
Treatments	**I**	**II**	**III**	**IV**	**V**	**VI**
LPD	0.1	0.7	4.3	16.2	5.7	10.6
HPD	0.08	0.6	2.3	18.3	6.4	8.6
LSD 5%	0.48**	0.37 NS	5.67 NS	22.29 NS	2.42 NS	4.56 NS

**Significant

Table 12: Effect of plant densities on fruit length, fruit diameter, yield, total dry root biomass and total dry biomass

		Variables			
Treatments	**Fruit length (cm)**	**Fruit diameter (cm)**	**Yield (kg/plant)**	**Total dry root biomass (g)**	**Total dry biomass (g)**
LPD	13.4	8.3	1.13	31.2	140.31
HPD	10.4	7.6	0.74	26.7	119.19
LSD 5%	5.47 NS	0.75 NS	0.32 NS	10.66 NS	23.45 NS

DISCUSSION

LPD treatment caused in important significant change in the leaf area index (LAI), at the final harvest. LPD treatment produced plants with more leaf per unit of ground area than HPD treatment and LPD treatment produced more total dry biomass and a higher yield than HPD. More leaf area increases biomass and therefore, a higher yield than HPD. That is, more leaf area increases the production of carbohydrates in the plant which is reflected in a higher production of biomass per plant and consequently a higher yield per plant compared to HPD. Also, plants under low population densities have less competition for water, light and nutrients, which may result in higher yields (Nesmith, 1998). Similar results were reported by Cushman et al. (2004) and Kultur et al. (2001) who indicated that low population densities in pumpkins and muskmelon can increase the size, weight and sugar content of fruits. Furthermore, crop growth can be considered as the product of incoming solar radiation, the fraction of that intercepted by the crop is determined by leaf area index (LAI), and the efficiency with which the intercepted radiation is used to produce biomass (Nam et al. 1998). Moreover, dry matter productivity of many crops can be linked with light interception (LI) (Muchow and Sinclair, 1994). Additionally, the amount of roots located in the first 10 cm (3.93 in) of soil did not show any significant difference between the treatments. In other words, the roots that help the plant hold the soil and prevent it from being eroded is the same for each treatment. This variable is very important for erosion prediction models, because the majority of the roots in most crops are between 0 to 10 cm depth (0 to 3.93 in). High populations increased competition for light, water and nutrients resulting in lower yield. Similar findings on competition for light and water nutrients were reported by Andrade et al.(1993), Cushman et al.(2004), Dutie et al.(1999a) and (1999b), Goldman (1995), Stofela (1996).

Data collected showed that high plant density (HPD) will produce taller plants and canopies. This result may indicate that at higher plant density, plant shoots are forced to grow taller in order to receive enough sunlight whereas, their roots grow deeper and wider in the soil to get nutrients and water in comparison to low plant density (LPD).

Data collected also indicated that LPD produced more roots than HPD. Similar findings are reported by Schulthesis et al., (1999) who indicated that low population densities in sweet potato accelerated root growth.

Overall, results show: Leaf area index and percent canopy cover, dry upper biomass were higher for LPD until the final destructive harvest. Root Mass 0-10 cm depth (dry weight) did not show any significant difference and total dry biomass (root+shoots) was higher (2.143 kg ha^{-1}) for LPD at the final destructive harvest compared to HPD (2.020 kg ha^{-1}).

These results contrast with the results for hot pepper, in which yield per plant decreased as plant population densities increased (Motsenbocker et al., 1997; Jovicich et al., 2004; Decoteau and Hatt, 1994).

Finally, these data provide a basis for future studies that may be focused on determining medicinal properties of eggplant, the use of high yielding varieties and different spacing that can generate recommendations for eggplant production soils in Mississippi.

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